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Cytogenetical Studies on the Intergeneric F_1 Hybrids between *Triticum Timopheevi* and 3 Species of *Secale*

By

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(Received August 20, 1954)

1. Introduction

Among the cytogenetical investigations on the wheat-rye intergeneric F_1 hybrids, those carried out on the hybrids between *T. Timopheevi* and *Secale* are very few in contrast to those hybrids among Dinkel or Emmer wheat and *Secale* species, and we see some paper published by Kostoff (1936, 1937) only.

As one of a series of cytogenetical studies on intergeneric hybrids between *Triticum* and *Secale*, the present author tried to make out some hybrids between *T. Timopheevi* and 3 species of *Secale* (*cereale*, *africanum* and *montanum*) in 1952, and obtained F_1 plants in crossing these combinations. Some cytogenetical studies on the F_1 plants were made and the results obtained will be dealt with in this paper.

2. Materials and methods

The seed of *S. africanum* and *S. montanum* used as the pollen parents in crossing experiments were kindly sent from Prof. Arne Muntzing of Lund University, Sweden. And the strain of *S. cereale* cultivated by the present writer since 1928 was employed.

The root tip cells and anthers fixed with Nawaschin's and Carnoy's fluid were used for the cytological study of somatic chromosomes and meiosis in the same method as in the previous paper by present author on wheat-rye hybrids. Original magnification of figures is $\times 3000$ for the somatic chromosomes and $\times 2300$ for the meiotic chromosomes.

3. Results of hybridization

The intergeneric hybridization between *T. Timopheevi* and each of the 3 species of *Secale* (*cereale*, *africanum* and *montanum*), was carried out in 1952, and the results obtained in these artificial crossings were summarized in the following Table 1.

As shown in Table 1, the seed setting in the hybrid between *T. Timopheevi* and *S. africanum* shows highest percentage and next comes that of the hybrid

Table 1. The results of hybridization between *T. Timopheevi*
and 3 species of *Secale*.

Combination	Number of spikes	Number of flowers pollinated	Number of grains obtained	Percentage of seed setting
<i>T. Timopheevi</i> × <i>S. cereale</i>	127	2455	1	0.041
<i>T. Timopheevi</i> × <i>S. africanum</i>	100	1888	417	22.034
<i>T. Timopheevi</i> × <i>S. montanum</i>	110	1700	291	17.118

Table 2. Germination of F_1 seeds.

F_1	Number of seeds sown	Number of seeds germinated	Percentage of germination	Number of plants died soon	Number of plants matured	Percentage of F_1 grown up to pollinated flowers
<i>TimoScF</i> ₁	1	1	100.00	0	1	0.041
<i>TimoSaF</i> ₁	100	79	79.00	10	69	15.292
<i>TimoSmF</i> ₁	291	19	6.53	5	14	0.824



Photo. 1. Spikes of *TimoScF*₁ and its parents, from left to right, *T. Timopheevi*, F_1 and *S. cereale*. × ca. $3/5 \times 2/3$



Photo. 2. Spikes of *TimoSaF*₁, *TimoSmF*₁ and its parents, from left to right, *T. Timopheevi*, *TimoSaF*₁, *S. africanum*, *TimoSmF*₁ and *S. montanum*. × ca. $3/5 \times 2/3$

between *T. Timopheevi* and *S. montanum* and that of the hybrid between *T. Timopheevi* and *S. cereale* the lowest.

The seeds thus obtained were sown in October of the same year and some F_1 plants were raised (Table 2).

It will be noticed here, that the seeds obtained in the third cross have germinated the lesser per cent than those of the second cross.

The difference between the percentage of seed setting in the second and third crosses was not so great as shown in Table 1, but it is significant in the percentage of the F_1 plants germinated in the two crosses. This seems due to the characteristic nature of the pollen parents. Similar results were also observed in crosses *T. persicum* \times *africanum* and *persicum* \times *montanum* (Nakajima, not yet published).

Lilienfeld and Kihara (1934) obtained some seeds corresponding to 17% for the number of pollinated flowers by artificial crossing between *T. Timopheevi* and *S. cereale*, but no F_1 plants were obtained, while Kostoff (1937) obtained 2 F_1 plants by the same combination.

The three kinds of hybrid raised by the present author will be represented as follows: *T. Timopheevi* \times *S. cereale* = *TimoScF₁*, *T. Timopheevi* \times *S. africanum* = *TimoSaF₁* and *Timopheevi* \times *S. montanum* = *TimoSmF₁*.

4. External characters of F_1 plants

Some individual differences were observed, though they were not so remarkable, in the external characters of the three kinds of F_1 plants. They are shown in the Table 3 and Photo. 1 together with the parental features.

Table 3. External characters of F_1 and its parental plants.

Characters Plants	Number of culms and spikes measured	Average length of culms cm	Average length of spikes cm	Average length of awns cm	Number of spikelets per spike	Spike density	Number of flowers per spikelet	Number of tillers
<i>T. Timopheevi</i>	25	136.08	5.22	7.52	22.00	4.28	3	
<i>S. africanum</i>	10	130.40	13.60	0.00	50.40	3.71	2	
<i>S. montanum</i>	20	110.00	15.23	1.23	44.60	2.93	2	
<i>S. cereale</i>	20	132.35	13.08	2.70	44.70	3.42	2	
<i>TimoScF₁</i>	10	133.50	10.55	6.85	28.90	2.64	4	236.00
<i>TimoSaF₁</i>	90	125.64	10.31	3.85	32.63	3.18	3	145.89
<i>TimoSmF₁</i>	50	128.12	11.61	4.58	31.62	2.73	4	152.80

As seen in Table 3 and Photo. 1, the culm heights the F_1 plants were intermediate of the parents except the *TimoSaF₁* which the case of the last mentioned F_1 plant, the length of culms is lower than both parents. And the length of spikes, awns and number of spikelets per spike of F_1 plants were intermediate of the parents in every combination. The number of flowers in

spikelet is superior to or same as that of the mother plant. But the number of spikelets per spike of the F_1 plants is somewhat decreased in every combination.

Generally speaking, though the F_1 plants possess external characters of both parents, they resemble more closely *T. Timopheevi*, the mother plant, than are intermediate (Photo. 1). Similar cases were previously reported by the present author in the F_1 obtained from *T. compactum* crossed with *S. cereale* (Nakajima 1950).

All these F_1 plants have thick hair on neck of the spikes. When ripened the spikes of *TimoSa* and *TimoSmF₁* become brittle, though less brittle than those of *TperSaF₁* plant (Nakajima, not yet published).

5. Seed fertility of F_1 plants

TimoScF₁: 236 culms with spikes were obtained from a single F_1 plant, and the spikes were normal in external characters but no pistil was found in all flowers. Similar cases were previously reported by Kostoff (1937) on the F_1 of *T. Timopheevi* × *S. cereale* and on the F_1 of *T. Timopheevi* × *Haynaldia villosa* by the present writer (Nakajima 1953). So far as is known pistils are not lacking in haploid plants, but in these cases it is not the case and it seems interesting to consider the fact that the F_1 plant lacks pistils.

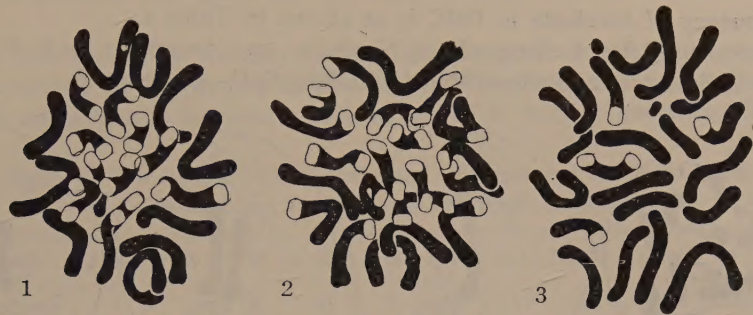
In consequence of the lack of pistils, the F_1 plant shows complete pollen sterility, while stamens have some fertility, for 5 grains of seed were obtained from ca. 6800 spikelets of the 236 spikes of the F_1 . The pollination of the stamens evidently took place with pollen of *MachScF₁* or *TperSaF₁* planted near by. The 5 seeds were sown in October 1953 and 2 plants were obtained and they are growing in spring of 1954.

TimoSmF₁: Of the 14 plants all flowers of every individual lack pistils completely as *TimoScF₁* described above, and consequently meiosis of PMC could not be observed.

TimoSaF₁: 69 F_1 plants has normal pistils, but the anthers were not generally opened and they were pollen sterile completely. In rare cases some anthers were opened, and 4 grains of seed were obtained from ca. 129000 spikelets on 3954 spikes of 69 F_1 plants cultivated separately from others. 2 of the 4 grains were injured by insects while straged, and the remaining 2 grains were sown in October 1953 and 2 F_2 plants were obtained.

6. Somatic chromosomes of F_1 plants

The somatic chromosome number of F_1 plants, raised by hybridization of the combinations mentioned above was 21 in every case (Figs. 1~3). This number corresponds to the sum of the gametic number of the parents.



Figs. 1~3. Somatic plates of root tip cells of *F*₁ plants. 21 chromosomes. × 2000. 1. *TimoScF*₁. 2. *TimoSaF*₁. 3. *TimoSmF*₁.

7. Maturation division in PMC's of *F*₁ plants

A. *TimoSaF*₁ In the present research, 69 *F*₁ plants (*TimoSaF*₁) were raised by crossing *T. Timopheevi* (*n*=14) with the pollen of *S. africanum* (*n*=7), but 9 individuals of them were actually used. In meiosis of PMC's the same number 21 was observed for the zygotic number (Figs. 4~13).

In heterotypic metaphase in the meiosis of PMC's univalents were scattered in spindle in most cases, but sometimes bivalents were also observed. The bivalents consist of two chromosomes of equal size, and in most cases stick-shaped bivalents in which two elements conjugated end to end loosely were observed, but as a rare case, formation of interstitial chiasma was observed, and, though rare, ring-shaped bivalent in which two chromosomes closely conjugated were also observed. The number of bivalents found in one PMC at metaphase in heterotypic division was 0~4 in 6 individuals among 9 of *F*₁, 0~5 in 2 of the remaining 3 individuals and 0~7 in the last one (Figs. 4~13). Consequently, 13~21, 11~21 and 7~21 univalents were observed respectively.

Table 4. Frequency of bivalents at heterotypic metaphase in PMC's of *TimoSaF*₁ plants.

Individuals	Bivalents and univalents								Mode (%)	Total
	0II +21I	1II +19I	2II +17I	3II +15I	4II +13I	5II +11I	6II +9I	7II +7I		
<i>TimoSaF</i> ₁ -1	216	113	101	62	8				0II(43.20)	500
2	208	103	106	71	12				0II(41.60)	500
3	225	132	93	43	7				0II(45.00)	500
4	177	84	125	75	31	8			0II(35.40)	500
5	207	118	104	51	20				0II(41.40)	500
6	167	112	170	104	41	6			2II(28.33)	600
7	216	129	151	96	69	28	8	3	0II(30.86)	700
8	203	128	98	53	18				0II(50.50)	600
9	259	155	123	52	11				0II(46.17)	600
Total	1978	1074	1071	607	217	42	8	3	0II	5000
%	39.56	21.48	21.42	12.14	4.36	0.84	0.16	0.06	39.56	100.02

Trivalent were counted as bivalents.

The frequency of bivalents in PMC is as shown in Table 4.

As seen in Table 4, the mode of bivalents was found to be 0 in 8 plants out of 9 individuals, and the mode of one, *TimoSaF*₁-6, being 2.



Figs. 4~14. Heterotypic division in PMC's of *TimoSaF*₁. $\times 920$. 4, Metaphase, 21 univalents scattered in spindle. 5, Side view of metaphase, 1II+19I. 6, do. 2II+17I. 7, do. 3II+15I. 8, do. 1III+2II+14I. 9, do. 4II+13I. 10, do. 5II+11I. 11, do. 1III+4II+10I. 12, do. 6II+9I. 13, do. 7II+7I. 14, do. 21 univalents splitting longitudinally.

Fig. 15. Several forms of conjugated chromosomes, a, bivalents, b, trivalents.

Trivalents were observed besides bivalents at the heterotypic metaphase, but no tetravalent was found. In most cases they were V-shaped, but sometimes chain-shaped (Fig. 15).

According to Lilienfeld and Kihara (1934) and Kostoff (1936, 1937) partial homology exist between G genome of *T. Timopheevi* and B genome of Emmer and Dinkel wheat. Also even the formation of 6 or 7 bivalents were observed to take place by the autosynopsis between A and B genomes of Emmer wheat as reported by Liljefors (1936) and Nakajima (1952).

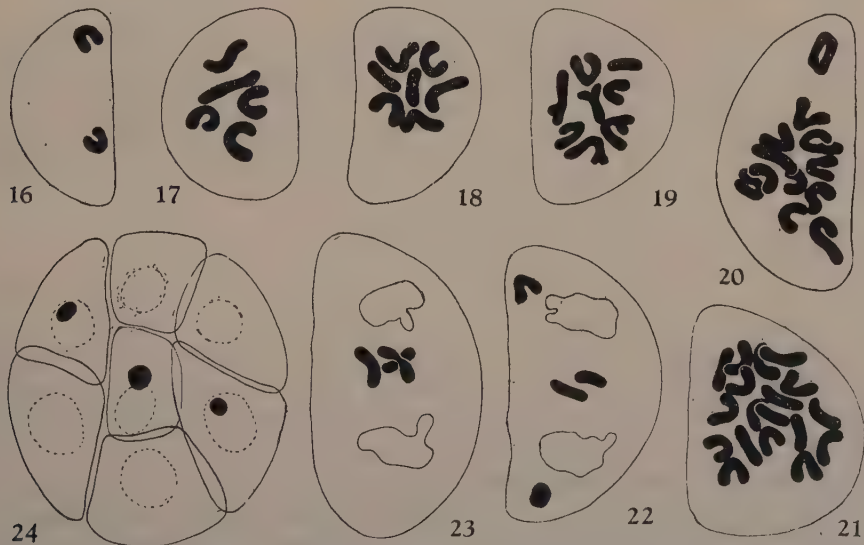
It may be said from these facts that the chromosomes of G genome and

those of A genome will give rise to some conjugations at any rate between them.

From this reasoning we may deduce that the greater part of at most 7 bi-valents observed by the author in the case of *TimoSaF*₁ might have been formed by the autosynopsis between chromosomes of A and G genomes, though of

Table 5. Distribution of chromosomes to the poles at the heterotypic ana-telophase in PMC's of *TimoSaF*₁ plants.

Individuals	2:19	3:18	5:16	6:15	7:14	8:13	9:12	10:11	Mode (%)	Total
<i>TimoSaF</i> ₁ -1	1		2	1	1	4	11	24	10:11 (51.00)	44
2		1	1	3	3	8	11	24	10:11 (40.00)	51
3	1	1	2	2	3	5	6	13	10:11 (39.39)	33
4			1	2	4	3	10	23	10:11 (53.49)	43
5			3		3	1	2	26	10:11 (74.29)	35
6				1	1	2	5	14	10:11 (60.87)	23
7	1		4	2	1	5	5	21	10:11 (53.92)	39
8		1		1	2	8	6	26	10:11 (59.09)	44
9				3	2	5	9	22	10:11 (53.66)	41
Total	3	3	13	15	20	41	65	193	10:11	353
%	0.85	0.85	3.68	4.25	5.67	11.61	18.41	54.68	54.68	100.00



Figs. 16~23. Homotypic division in PMC's. ×920. 16, Polar view of metaphase 2 chromosomes. 17, do. 5 chromosomes. 18, do. 7 chromosomes. 19, do. 8 chromosomes. 20, do. 12 chromosomes, 21, do. 18 chromosomes. 22, Side view of ana-telophase, 2 lagging chromosomes. 23, do. 3 lagging chromosomes.

Fig. 24. Heptad in tetrad stage, showing a nucleolus in each of 3 cells of it,

course a bivalent produced by the autosynopsis between chromosomes of A genome itself.

The distribution of chromosomes to opposite poles at the ana-telophase in heterotypic division proceeded at random, viz., the cases of 10:11 and 19:2 were observed, but in most cases, the number of chromosomes distributed to the poles was usually not so different in two groups. The frequency of chromosome groups of unequal chromosome distribution are as shown in Table 5.

As is evident from Table 5, the frequency of chromosome distribution of such proportions as 10:11~8:13 was relatively high and was found to be 84.7%. Consequently that of the remaining cases, 7:14~2:19 showed very low percentage 15.3%.

All the univalents showed longitudinal splitting at the metaphase of heterotypic division and consequently the restitution nucleus was formed.

In the stage after homotypic division, the nuclear division was proceeded more or less irregularly as in many *Triticum-Secale* F_1 hybrids, consequently lagging chromosomes were observed generally.

Table 6. Number of cells in the "tetrad stage of PMC's" of *TimoSaF₁* plants.

Number of cells Individuals	4	5	6	7	8	Mode	Total
<i>TimoSaF₁</i> -1	75	15	9	1		4	100
2	75	18	7			4	100
3	77	8	13	2		4	100
4	79	16	4	1		4	100
5	76		17	3	4	4	100
6	72	4	24			4	100
7	70	6	23	1		4	100
8	77	6	17			4	100
9	70	6	24			4	100
Total	671	79	138	8	4	4	900
%	74.56	8.78	15.33	0.89	0.44	74.56	100.00

In the tetrad stage, the number of cells varied in range of 4~8 in most cases (Table 6). The case of one consisted of 4 cells was shown to be the mode (74.56%). The tetrads have normal shape and micronuclei resulted from irregular division were often included in the tetrads.

B. *TimoScF₁* and *TimoSmF₁* In the F_1 plants, raised from two combinations of hybridization between *T. Timopheevi* and *S. cereale* or *montanum*, the meiosis of PMC's could not be observed, for the pistils were lacking completely in every individual as mentioned above in the section 5: fertility of F_1 plants.

One individual of 2 F_1 plants, obtained by Kostoff (1937) by *T. Timopheevi* × *S. cereale*, reported to have normally developed anthers, while in the case of the other one he noted that the anthers degenerated at a very early stage of development. Further, he noticed that usually 21 univalents or 19 univalents

with one bivalent and in a few instance with 2 bivalents were observed in the heterotypic metaphase of PMC's of normal F_1 plant.

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Studies on the Formation of Ascorbic Acid (Vitamin C) in Plants

6. Relation among Formation of Ascorbic Acid, Development of Chlorophyll and Beginning of Photosynthesis

By

Tomota SUGAWARA

(Received August 31, 1954)

Introduction

There are published many observations that the ascorbic acid content of plants is affected by weather factors such as light intensity. It is generally believed that the ascorbic acid content of leaves is particularly high under the high activity of photosynthesis.

In the previous paper the author has reported the fact that the etiolated seedlings exposed to low light intensity contained considerably less ascorbic acid than those exposed to high light intensity, and that the difference in the ascorbic acid content of seedlings under the two light conditions was practically attributable to the difference of light intensities. It was further stated that when seedlings containing chlorophyll were exposed to light, ascorbic acid was formed in their leaves, so that direct relationship between the activity of photosynthesis and the quantity of ascorbic acid may be quite probable except during germination period.

Inman et al., on the other hand, reported that chlorophyll a was formed first and chlorophyll b a little later, and that photosynthesis began after the formation of chlorophyll b. Goodwin and Owens found that chlorophyll b followed the formation of chlorophyll a by about three hours when etiolated oat seedlings were exposed to light.

This paper is concerned with exploratory studies of the relation among formation of ascorbic acid, development of chlorophyll and beginning of photosynthesis.

Materials and methods

Helianthus annuus L., *Zea Mays* L., *Brassica Rapa* L., *Vicia Faba* L., and *Avena sativa* L. were employed in this experiment. Seeds of these plants were germinated in quartz sands at 25°C in the air-conditioned darkroom. When germinated, all seedlings were grown in the darkroom at about 20°C for 10 days and then these etiolated seedlings were continuously exposed to light at 2,000 Lux. During the illumination, the content of ascorbic acid, chlorophyll a and b, and, the evolution of oxygen by photosynthesis were measured at intervals of

15-40 minutes. The experiments were usually conducted in duplicate, and repeated at least twice.

The amount of ascorbic acid was determined by the titration method with 2, 6-dichlorophenolindophenol solution, as described in previous reports. The chromatographic adsorption technique was used to separate and isolate the green pigments. Further in order to identify the pigments involved, their absorption spectra were taken by means of a Beckman spectrophotometer. Seedlings were tested for the liberation of oxygen before exposure to light and every 15 minutes for a period of 220 minutes. Luminescent bacteria were used to detect the presence of oxygen.

Results and consideration

A number of seedlings 10 days old were continuously exposed to light, and chlorophylls and ascorbic acid were then measured every 15-30 minutes. The results of determinations are shown in Table 1.

Brassica Rapa L., and *Helianthus annuus* L., showed variations of from 30 to 60 minutes by the time before the first appearance of chlorophyll in the leaves of seedlings. However, the liberation of oxygen always began almost at the

Table 1. Formation of ascorbic acid, chlorophyll a and b, and beginning of photosynthesis.

Time (Minute)	<i>Zea Mays</i> L.			<i>Brassica Rapa</i> L.			<i>Helianthus annuus</i> L.		
	Appear- ance of chloro- phyll	Evolu- tion of oxygen	Content of total ascorbic acid	Appear- ance of chloro- phyll	Evolu- tion of oxygen	Content of total ascorbic acid	Appear- ance of chloro- phyll	Evolu- tion of oxygen	Content of total ascorbic acid
15	—	—	(mg/g) 0.501	—	—	(mg/g) 0.556	—	—	(mg/g) 0.482
30	—	—	0.496	(a)	—	0.560	—	—	0.476
60	(a)	—	0.511	a	+	0.542	—	—	0.485
90	a	+	0.501	a	+	0.556	(a)	+	0.482
120	a	+	0.494	a, (b)	+	0.549	a	+	0.491
160	a, (b)	+	0.507	a, b	++	0.571	a	+	0.498
190	a, b	++	0.549	a, b	++	0.642	a, (b)	+	0.544
220	a, b	+++	0.602	a, b	+++	0.717	a, b	++	0.613

same time when one could detect a faint appearance of a greenish color in the leaf. In etiolated seedlings, however, chlorophyll a was formed much more rapidly than chlorophyll b, when the plants were first exposed to light, regardless of the spectral region employed.

Although not specifically commenting on this interesting phenomenon, Frank has recently published spectrophotometric data indicating that the same thing holds true for completely dark-grown seedlings of *Avena byzantium* exposed five hours to light. It is possible that this early rapid production of chlorophyll a may be correlated with the initial presence in etiolated seedlings of a relatively higher percentage of protochlorophyll a than of protochlorophyll b.

In the present experiments, the author has observed an almost complete absence of chlorophyll b in chlorophyll extracts obtained from plants radiated for 120 or 160 minutes with visible light. Fig. 1 shows the relative absorption per leaf of extracts from completely dark-grown plants in a spectral region. The two absorption maxima for the dark-grown plant extract probably represent the absorption of protochlorophyll and of chlorophyll a.

Figs. 2 and 3 show the relative absorption per leaf of extracts from irradiated plants in a spectral region. A typical chlorophyll absorption spectrum was obtained after 190 minutes exposure to the light at 1,000 Lux.

The amount of ascorbic acid does not increase till the beginning of photosynthesis after the formation of chlorophyll, but the chlorophyll b is not essential for photosynthesis. The results show that formation of ascorbic acid has a close correlation with photosynthesis, i.e., ascorbic acid is formed only after the beginning of photosynthesis. In general, however, the formation of ascorbic acid in seeds slightly becomes active in the

early stage of germination and then shows a rapid increase until the nutrient is apparently exhausted and thereafter the amount of ascorbic acid decreases in germinated seeds. Such tendency has already advanced the view that the formation of ascorbic acid in seeds promoting the germination process before the beginning of photosynthesis.

From the results of this experiment, it may certainly be stated that formation of ascorbic acid, appearance of chlorophyll a, and beginning of photosynthesis stand in a close physiological interrelationship.

The ratio of chlorophyll a to chlorophyll b has been determined for the green tissue of many species of plants. Willstätter studied many green plants and found, on an average, the ratio of about three parts of chlorophyll a to one part of b. Comar and Frank reported that the chlorophyll a usually lies between 67%

and 78% for the normal green tissues of higher land plants. The relation between the above ratio and ascorbic acid content is shown in table 2.

The results show that when the etiolated leaves of *Avena sativa* and *Vicia Faba* are irradiated for one hour and the first evolution of oxygen is readily detected by use of luminous bacteria, the chlorophyll b is not yet formed even in a trace. When the seedlings were irradiated two hours more, the value of

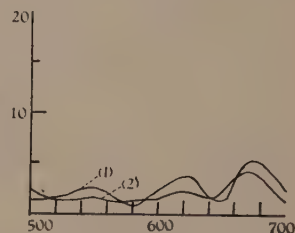


Fig. 1. Relative absorption spectra of extracts of leaves from completely dark-grown seedlings.

- (1) *Brassica Rapa* L.,
(2) *Zea Mays* L.

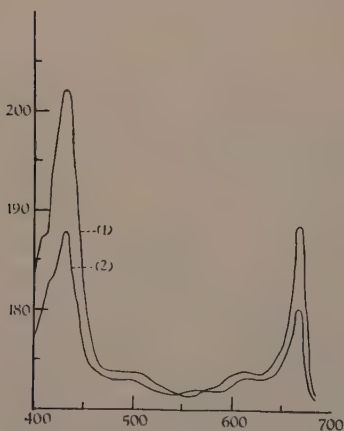


Fig. 2. Relative absorption spectra of chlorophyll a extracts of leaves from irradiated seedlings.

- (1) *Brassica Rapa* L.,
(2) *Zea Mays* L.

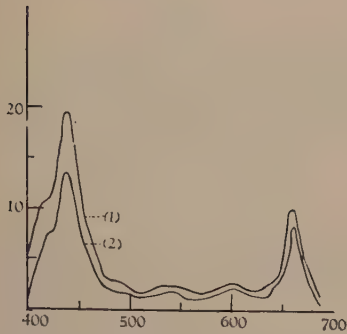


Fig. 3. Relative absorption spectra of chlorophyll b extracts of leaves from irradiated seedlings.

- (1) *Brassica Rapa* L.,
(2) *Zea Mays* L.

the ratio of a to b was found to be about 16-19. This indicates that the normal ratio of three parts of a to one part of b is not essential for photosynthesis, if one assumes that the process of photosynthesis is certainly under way when the evolution of oxygen can be detected. No attempt was made to test the absorption of carbon dioxide during the experiments. The ratio of chlorophyll a to chlorophyll b is not indispensable for the beginning of photosynthesis and the formation of ascorbic acid has a close relation with photosynthetic activity than the ratio of chlorophyll a to b.

Other experiments with *Porphyra tenera* Kjellman having only chlorophyll a, showed the content of 38.25 mg. ascorbic acid in 100 g.

Table 2. Ratio of chlorophyll a to b and content of ascorbic acid.

Time (Minute)	<i>Avena sativa</i> L.				<i>Vicia Faba</i> L.			
	Leaf color	Chloro- phyll a:b	Evolution of oxygen	Content of total ascorbic acid (mg/g)	Leaf color	Chloro- phyll a:b	Evolution of oxygen	Content of total ascorbic acid (mg/g)
0	Yellow	—	—	0.560	Yellow	—	—	0.473
30	Yellow green	a only	—	0.551	Yellow green	a only	—	0.481
60	Yellow green	a only	+	0.580	Yellow green	a only	+	0.496
120	Light green	19:1	+	0.620	Green	18.7:1	+	0.610
220	Green	18.2:1	++	0.698	Green	16.3:1	++	0.692

living tissues. But it can perform photosynthesis of course. Formation of ascorbic acid, therefore, is not influenced by the content of chlorophyll b, but by photosynthesis.

Goodwin and Owens observed that in etiolated oat seedlings, when exposed to light, chlorophyll a was produced, and only after about three hours chlorophyll b was detected. Highkin studying a barley mutant, chlorina #2, which was capable of photosynthesizing, reported that it seemed entirely lacking in chlorophyll b, although its chlorophyll a content was normal. That chlorophyll b is not essential for initiation of photosynthesis and formation of ascorbic acid may be suggested by these experiments.

Conclusion

Leaves of dark-grown seedlings become green by illumination, and only after about two or three hours chlorophyll b was detected. Chlorophyll b is not essential for the initiation of photosynthesis and the formation of ascorbic acid,

although chlorophyll b is, without exception, contained in the leaves of normal green land plants.

From the results of these experiments, it may certainly be stated that the formation of ascorbic acid has a close connection with the initiation of photosynthesis, but has not direct connection with the appearance of chlorophyll b.

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On the Germination of the Pollen-grains of Ericaceae

By
Atsushi KUBO

The problem of physical and physiological dryness.

by Y. Fukuda. Report 13.

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Molisch (1893) succeeded in making the pollen-grains of Ericaceae germinate in water in which either a pistil or malic acid had been put. This method was approved by Ridforss (1896), and was improved by von Berg (1929) who added nicotin to the malic acid medium. According to Sasaki (1919) the germination ratio of the pollen-grains of *Rhododendron obtusum* Planch. on the medium of 1.0% agar and 10.0% sucrose was 4.4%. Hitherto no one has obtained the normal germination ratio of Ericaceae and of some other families like Compositae, of which, however, *Cosmos* has lately been studied and reported by the present author (1954). Another investigation in this direction on Ericaceae will now be published in this paper.

Material and Methods

The series of the experiments were carried out at Tagawa Branch of Fukuoka Liberal Arts College. The pollen-grains of *Rhododendron obtusum* Planch., *R. obtusum* var. *Kaempferi* Wils., *R. lateritium* Planch. and *R. dilatatum* Miq. were used; agar, gelatin or gum arabic was used to prepare media in which malic acid, nicotin or sucrose were added. Both thin (10 μ) and thick (2 mm) layers were used. Buffer solution used for the adjustment of pH of the medium is α -solution of Britton & Robinson: a certain amount of N/5 NaOH to 100 cc. of acid mixture (Mol/25 phosphoric acid, acetic acid and boric acid in 1000 cc. of solution). An equal amount of buffer solution was added to the medium prior to the application. Having been scattered on the preparation, the pollen-grains were kept in a moist chamber at room temperature.

Experimental Results

Exp. 1. **Effect of malic acid and nicotin.** As denoted on Table 1, in a shallow layer of malic acid solution pollen-grains germinated within the range of 0.06% and 0.3%, and in a deep layer within 0.0038% and 0.05%; and the germination ratio in the latter medium was better than in the former. The highest germination ratio after the device of Molisch was 40% (Table 1). No grain germinated on 0.03~1.7% nicotin solution.

The medium used by von Berg consists of 0.01~0.5% malic acid and 1.0% nicotin, and in it the author could get only 13% germination at the best.

Table 1. Variation of germination ratio (%) of pollen-grains of *Rhododendron obtusum* caused by the change in the depth of a layer of malic acid or nicotin solution.

Malic acid	May 31	Concentration of acid	3.8	5.6	7.5	10.0	15.0	17.5	20.0	22.5	(mg. in 100 cc.)					
		Shallow Deep	0 0	0 0	0 6.0	0 3.5	0 0	0 0	0 40.0	0 0						
		Concentration of acid	25.5	30.0	35.0	40.0	45.0	50.0	60.0	(mg. in 100cc.)						
		Shallow Deep	0 0	0 14.0	0 0	0 15.0	0 0	0 2.5	13.3 0							
Nicotin	June 18	Concentration of acid	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.1	1.3	1.5	1.7%	
		Shallow Deep	0 0	3.5 0	0.2 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	
	June 29	Concentration of nicotin	0.03	0.06	0.13	0.25	0.5	1.0	1.3	1.5	1.7	%				
		Shallow Deep	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0						
10% nicotin & malic acid	June 29	Concentration of acid	0.03	0.06	0.13	0.25	0.5	1.0	1.3	1.5	1.7	%				
		Shallow Deep	0 3.0	5.0 13.0	0.2 10.0	0 3.0	0 0	0 0	0 0	0 0	0 0					

Table 2. Variation of germination ratio (%) of pollen-grains of *Rhododendron obtusum* in gelatin caused by the change in the malic acid content thereof.

Medium	Layer	Concentration of malic acid (mg. in 100 cc.)												Date	
		35.0	40.0	45.0	50.0	60.0	80.0	90.0							
Gelatin 6.0% & Sucrose 5.0%	Thin	28.0	41.9	85.0	80.0	60.0	38.0	42.5	%						May 31
	Thick	100	100	100	100	100	100	100							
		Concentration of Malic acid (%)													
		0.1	0.2	0.3	0.4	0.5	0.7	0.9	1.1	1.3	1.5	1.7			
	Thin	46.5	37.0	61.0	100	100	100	96.0	11.8	0	0	0			
	Thick	50.8	46.0	0	0	0	0	0	0	0	0	0			
Gelatin 12.5% & Sucrose 5.0%	Thin	10.0	25.0	4.5	23.0	0	0	0	0	0	0	0	June 12	June 18	
	Thick	12.9	2.0	1.3	0.1	0	0	0	0	0	0	0			
	Thin	14.5	5.5	30.0	0	0	0	0	0	0	0	0			
	Thick	3.5	2.5	0	0.3	0	0	0	0	0	0	0			

Exp. 2. Improving germination by adding gelatin to malic acid solution. Six % gelatin and 5% sucrose (Table 2) were added to the malic acid solution

lower than 0.1%, and 100% germination ratio was obtained on a thick layer of this medium. When the concentration of malic acid was higher than 0.1%, harmful effect of the acid appeared. This harmful effect was strong in a thick layer. The increase in gelatin concentration to 12.5% (June 12, 18) lowered the germination ratio and narrowed the germination range of malic acid concentration.

Table 3. Germination ratio (%) of pollen-grains denoting the different fitness of gelatin and agar. (No buffer in the medium; thick layer; sucrose 10.0%.)

Date	May 8	17	17	9	9	9	
Medium	Gelatin & sucrose	Gelatin & sucrose	Gelatin	Gelatin	Agar	Agar & sucrose	Species
Concentration of gelatin or agar (%)	12%	100					<i>R. lat.</i>
		100					<i>R. obs.</i>
		100					<i>R. o. Kaem.</i>
	9%	100	100	100	27.0		<i>R. l.</i>
		100	100	100	100		<i>R. o.</i>
		100	100	100	96.0		<i>R. o. K.</i>
	7%	100	100	100	30.0		<i>R. l.</i>
		100	100	100	100		<i>R. o.</i>
		100	100	100	98.0		<i>R. o. K.</i>
	6%	100	100	100	100		<i>R. l.</i>
		100	100	100	100		<i>R. o.</i>
		100	100	100	100		<i>R. o. K.</i>
	5%	100	100	100	100		<i>R. l.</i>
		100	100	100	100		<i>R. o.</i>
		100	100	100	100		<i>R. o. K.</i>
	4%	100	100	99	100	46.2	<i>R. l.</i>
		100	100	100	100	21.8	<i>R. o.</i>
		100	100	100	100		<i>R. o. K.</i>
	3%	100	100	100	100	26.7	<i>R. l.</i>
		100	100	100	100	30.0	<i>R. o.</i>
		100	100	100	100		<i>R. o. K.</i>
	2%				0.4	3.9	<i>R. l.</i>
					30.0	2.2	<i>R. o.</i>
	1%					0	<i>R. l.</i>
						0	<i>R. o.</i>

Exp. 3. **Specificity of agar and gelatin.** The threshold concentration of plasmolysis of pollen-grains was estimated to be similar to that of 35% sucrose. In the medium of lower concentration than 35% sucrose or 6% gum arabic, the

membrane of pollen-grains remained unchanged but the plasma belched out. From these facts we know that hypertonic solution causes plasmolysis of grains and hypotonic one injures plasma, and there is no suitable hydrature state for germination in sucrose solution. Exp. 2 showed that addition of gelatin improved this character of liquid medium, but in this experiment it seemed that gum arabic can not be substituted for gelatin. On the agar medium of higher concentration than 4.0%, the pollen-grains could hardly attach to the surface of medium. On the gelatin medium of lower concentration than 2.0%, the pollen-grains were embedded in the medium. Therefore the media with agar less than 4.0% or with gelatin more than 2.0% were employed (Table 3).

The experiment was made only on thick layers. On the gelatin medium, all the pollen-grains of *R. obtusum* and *R. obtusum* var. *Kaempferi* germinated perfectly and all of *R. lateritium* did nearly perfectly, too. But the germination ratio a little declined on the gelatin medium of 7.0% and 9.0% and became zero at 12.0%. The addition of sucrose improved the germination ratio (May 17) and raised it to 100% even in the 12.0% gelatin medium. It is presumed that sucrose which absorbs water from air humidity improves the germination ratio which has decreased because of the larger content of gelatin. Such success was achieved only on the thick media, while all experiments failed on the thin media. The similar phenomenon was observed also on untimely flowers (Exp. 4. Table 4.).

Exp. 4. **Untimely early flowers in January, 1954.** *R. dilatatum* bloomed in January owing to the unusual warmth (Table 4.).

Table 4. Germination ratio and the length of pollen-tubes of untimely flowers (January, 5°C) of *R. dilatatum*. Thick layer without buffer.

Time after sowing			hours	4	5	6	7	8	9	13	22	120
Temp.	Medium		Germination ratio (%)									
18°C	Gelatin	8.0%	0.	0	0	0	0	0	0	0	0	0
"	Gelatin	4.0	70.0	85.0	85.0	98.0	98.0	98.0	98.0	100	100	100
"	Agar	1.0	0	0	0.1	0.1	0.1	0.1	0.1	8.0	13.0	13.0
"	Agar	0.5	0	0	0	0	0	0	0	8.0	8.0	8.0
5°C	Gelatin	4.0	0	0	0	0	0	0	0	0	0	6.0
			Pollen-tube length (μ)									
18°C	Gelatin	4.0%	62	74	148	197	246	277	984	1020	1130	
"	Agar	1.0			25	49	52	123	443	615	763	
"	Agar	0.5							185	467	677	
5°C	Gelatin	4.0										148

At the room temperature (5°C) the pollen-grains germinated very slowly. All experiments failed excepting that in the gelatin medium of 4.0%, 6.0% of grains germinated after 120 hours. But at 18°C in an incubator they completely germinated in 4.0% gelatin medium after 13 hours. Eight % medium was too stiff. In agar only a small number of grains germinated. On a thin layer of agar no germination took place even when butter solution was added.

Exp. 5. **Variation of refraction of pollen-grains caused by the change in the water content thereof.** Pollen-grains looked dark when they were dry, but they became transparent when they took in water. Therefore the water content of pollen-grains may be observed by a microscope. This particular refraction of pollen-grains, which appears when they absorb water, was not observed in nicotin solution even though it was dilute (Exp. 1, Table 1).

Table 5. Germination ratio in concentrated medium of agar or gelatin.

Date	May 14						June 3			
Medium	Agar 1.0%		Gelatin 4.0%		Gelatin 6.0%		Gelatin 4.0%	Agar 0.5%		
Sucrose	10%		10%		0		10%	5.0%		
Layer	Thin	Thin	Thick	Thin	Thick	Thin	Thin	Thin	Thin	Thin
Pollen age	Young	Old	Young	Young	Young	Young	Young	Old	Young	
	pH 1.8						<i>R. lateritium</i>			
<i>R. lat.</i>			0	0	0	0	Red	6.1	0	3.3
<i>R. obs.</i>	0	0			0	0	Pink	2.0	17.3	6.0
<i>R. o. Kaem.</i>			0	0	0	0				
	pH 2.8									
<i>R. l.</i>			0	0	0	0	Red	5.7	0	0
<i>R. o.</i>	0	0			0	0	Pink	72.5	28.5	20.0
<i>R. o. K.</i>			0	0	0	0				
	pH 3.8									
<i>R. l.</i>			0	0.3	0	0	Red	3.2	0	1.5
<i>R. o.</i>	9.2	0			0	0	Pink	40.0	9.3	40.0
<i>R. o. K.</i>			0	0	0	0				
	pH 4.8									
<i>R. l.</i>			0	0	0	0	Red	0.3	0	10.6
<i>R. o.</i>	1.0	0			0	0	Pink	47.3	0	2.7
<i>R. o. K.</i>			0	5.0	0	0				
	pH 5.8									
<i>R. l.</i>			0	0	0	0	Red	0	0	0
<i>R. o.</i>	0	10.0			0	0	Pink	4.2	0.2	0
<i>R. o. K.</i>			0	0	0	0				
	pH 6.8									
<i>R. l.</i>			0	0	0	0	Red	0	0	0
<i>R. o.</i>	0	0.1			0	0	Pink	0	0	0
<i>R. o. K.</i>			0	0	0	0				
	pH 7.8									
<i>R. l.</i>			0	0	0	0	Red	0	0	0
<i>R. o.</i>	1.0	0			0	0	Pink	0	0	0.2
<i>R. o. K.</i>			0	0	0	0				

The germination ratio in Exp. 5 (Table 5) on June 3 increased more than that of May 14 owing to the rise of temperature. The similar result was obtained in Exp. 3. In the experiment 5 the pollen-grains in a concentrated medium did not look so transparent as those in a dilute one. The similar phenomenon

was also observed in Exp. 2 of May 9 (Table 3). This observation proves that, as solute, colloid substance such as gelatin and agar, physically restricts the water suction of pollen-grains (Table 5), and that nicotin physiologically inhibits it (Table 1).

Exp. 6. **Influence of ions in buffer solution.** When buffer solution was added to the medium (Table 6), all the results of experiments on thick layers were negative, and only positive results on thin layers are illustrated on Table 6.

Table 6. Germination ratio in 1.0% agar or 4.0% gelatin of different hydrogen ion concentrations. The layer thin; sucrose 10.0%. An equal volume of buffer solution was added to the medium.

Date	May 9		14		9		June 1		18		May 18			
Medium	Gelatin		Gelatin		Agar		Agar		Agar		Agar			
Sucrose	4.0%		10%		1.0%		0		0		10%			
Species	<i>R. l.</i>	<i>R. o.</i>	<i>R. l.</i>	<i>R. o.</i>	<i>K.</i>	<i>R. l.</i>	<i>R. o.</i>	<i>R. l.</i> Pink	<i>R. l.</i> Red	<i>R. l.</i> Pink	<i>R. l.</i> Red	<i>R. l.</i>	<i>R. o.</i>	<i>K.</i>
pH	1.8	0	0	0	0	0	0.3	0	0	10.0	30.0	0	0	
	2.8	1.0	0	1.0	0	0	0	14.1	3.5	0	30.0	1.0	5.0	
	3.8	0	0	0	5.0	0	0	0	0	14.0	9.0	3.3	0	
	4.8	1.6	0	0	0	4.5	0	0	0	0.7	6.0	0.3	0.5	
	5.8	0	0	0	0	1.0	0.5	0	0	0.7	0	2.0	0	
	6.8	0	0	0	0	0	0	0	0	0	0	1.0	2.3	
	7.8	0	0	0	0	0	0	0	0	0	0	0	0	
	8.8	0	0	0	0	0	0	0	0	0	0	0	0	
	9.8	0	0	0	0	0	0	0	0	0	0	0	0	
	10.8	0	0	0	0	0	0	0	0	0	0	0	0	

The influence of ions was so strong that the pollen-grains did not germinate on the thick layer, while on the thin layer germination took place at pH below 7.8. Salts in buffer solution perhaps absorb vapour in a moist chamber, and improve germination condition on a thin but not thick layer.

Exp. 7. **Effect of different amount of buffer solution.** To the medium with 2.0% agar and 10.0% sucrose buffer solution was added as follows, in **a** 2:1, **b** 1:1 and **c** 1:2. The results of this experiment are shown in Table 7, and we see that **a** was better than **b** or **c**. The less buffer solution, the better was germination. The fact indicates that salt ions are harmful. The harmful effect lessens as pH value lowers below 7.8. The pollen-grains of Ericaceae seem not to absorb water in an extremely high or low hydrogen ion concentration. The total effect of many factors in medium such as colloidal substance, sugar, salt and hydrogen ion concentration is no other than the effect of hydrature equilibrium between grains and medium.

Exp. 8. **Time function on germination.** The experiment was made on June 10 when the temperature was favourably high for germination. *R. lateritium* was used. The degree of extension of the pollen-tubes was measured. The

pollen-grains of red flower variety were used for culture both in fresh and dried condition (on CaCl_2 for 6 hours). But those of pink flower variety were used fresh. Though the plants were in full bloom in May, the pollen-grains did not germinate owing to the unfavourable climate. In June, the germination

Table 7. Germination ratio in various proportion of buffer solution in the medium (Agar 1.0%, sucrose 10.0%).

Date	June 2						June 9		
Buffer solution to medium	M.2:B.1 a		M.1:B.1 b		M. $\frac{1}{2}$:B.2 c		M.2:B.1 a	M.1:B.1 b	M. $\frac{1}{2}$:B.2 c
Layer	Thin	Thick	Thin	Thick	Thin	Thick	Thin	Thin	Thin
Species	pH 1.8								
<i>R. l.</i> (Red)	0	5.7	0	0	0	3.3	60.0	0.4	0
" (Pink)	0	100	0	0.9	0	6.0	80.0	16.4	11.0
<i>R. o. K.</i>		100							
	pH 2.8								
<i>R. l.</i> (Red)	0	5.9	0	0	0	0	80.0	0	0
" (Pink)	9.5	100	0	11.5	0	20.0	100	0	0
<i>R. o. K.</i>		100							
	pH 3.8								
<i>R. l.</i> (Red)	0	5.9	0	16.7	0	1.4	100	0	0
" (Pink)	0	100	0	0	0	40.0	100	0	0
<i>R. o. K.</i>		100							
	pH 4.8								
<i>R. l.</i> (Red)	2.6	1.2	0	1.5	0	10.6	2.0	0	3.0
" (Pink)	100	18.8	3.5	8.0	0	2.7	30.0	0	18.5
<i>R. o. K.</i>		2.0							
	pH 5.8								
<i>R. l.</i> (Red)	0	0	0	0	0	0	7.0	0	0
" (Pink)	0	5.0	0	0	0	0	22.0	0	0
<i>R. o. K.</i>		24.0							
	pH 6.8								
<i>R. l.</i> (Red)	0	0	0	0	0	0	0	0	0
" (Pink)	0	0	0	0	0	0	42.9	0	0
<i>R. o. K.</i>									
	pH 7.8								
<i>R. l.</i>	0	0	0	0	0	0	0	0	0
" (Pink)	0	0	0	0	0	0.2	4.0	0	0
<i>R. o. K.</i>		0							

ratio became 100% in a lower concentration of agar (0.6%) and sucrose (10.0%) though it took 24 hours for germination. (Table 8, 9, 10). Exp. 5 also shows that June was better than May. On a thick layer the pollen-grains germinated better in higher concentrations. As the thin layer dried easily when it was

taken out of the moist chamber for the measurement of tube length, the pollen-tube elongation on it was checked, though the germination ratio reached 100% in the moist chamber after 24 hours. During that time, the medium absorbed

Table 8. Germination ratio and length of pollen-tubes. Pink flower variety of *R. lateritium*. Thick and thin layers. Agar concentration: 2.0%, 1.0% and 0.6%; Sucrose: 10.0%.

Time after sowing		hours 2.5		3		4		5		6		24	
Pollen condition		Dry	Fresh	Dry	Fre.	Dry	Fre.	Dry	Fre.	Dry	Fre.	Dry	Fre.
Layer	Agar	Germination ratio (%)											
Thick	2.0%	0	0	0	0.1	2.0	2.0	3.0	5.0	20.0	11.4	30.0	100
"	1.0	0	0	0.1	4.4	2.0	6.0	4.0	11.0	5.0	12.0	20.0	100
"	0.6	0	0	0	2.1	0	4.7	0	35.0	0	48.9	4.0	100
Thin	2.0	0	0	0	0	0	0	0.1	2.0	0.1	2.8	20.0	100
"	1.0	2.0	0	3.3	0	8.8	0	9.2	3.0	14.0	3.5	16.0	100
"	0.6	0	0	0	0	0	0	0	0	0	0	5.0	100
		Tube length (μ)											
Thick	2.0				48	62	48	70	53	73	59	430	984
"	1.0			44	44	62	53	64	98	62	148	304	1968
"	0.6				87		90		102		120	74	2460
Thin	2.0							25	25	33	28	344	492
"	1.0	74		38		73		85	37	90	41	320	492
"	0.6											148	168

Table 9. Germination ratio and length of pollen-tubes of red flower variety of *R. lateritium*. Thick and thin layers. Agar added to different concentrations to 10.0% sucrose.

Time after sowing		hours 2.5	3	4	5	6	24
Layer	Agar	Germination ratio (%)					
Thick	2.0%	0	6.0	11.0	18.0	21.0	100
"	1.0	0.1	20.0	30.0	40.0	45.0	100
"	0.6	18.0	30.0	24.0	40.0	100	100
Thin	2.0	0	0	0	0	0	0
"	1.0	0	0	0	0	0	0
"	0.6	0	0	3.0	3.5	3.5	100
		Tube length (μ)					
Thick	2.0%		41	59	74	89	1203
"	1.0	48	66	123	185	548	2792
"	0.6	76	112	148	656	790	3280
Thin	0.6			67	73	73	1476

vapour from the air of the moist chamber, and because of the increase in hydrature the elongation of pollen-tubes became faster but that of dry pollen-grains did not. The higher the concentration of the solute is, the more favourable the hydrature condition becomes for fresh pollen-grains, but dry ones die because of the rapid suction of water.

Exp. 9. **Effect of growth periodicity and pH.** Every two or four days during the flowering season of *R. lateritium* (Table 10) the germination ratio of pollen-grains was observed. At the beginning of the flowering season very few flowers were in bloom, and most of the flowers bloomed suddenly toward the end of the second week. Life duration of each flower was five days in average, and the flowering season continued for several weeks. The samples of pollen-grains were taken from a flower when it is in its most vigorous stage. In

Table 10. Shifting of optimum pH for pollen-grain germination which was brought about by the growth and seasonal changes in *R. lateritium*. Thin layer. An equal amount of the medium (1.0% agar and 10.0% sucrose) and of the buffer solution were mixed. Control: thick layer of medium (2.0% agar, 20.0% sucrose; 1.0% agar, 10.0% sucrose) without the addition of buffer solution.

Species	<i>R. lateritium</i> (red flower)									Do. (pink flower)				
Date	June	1	3	5	9	10	14	18	23	1	3	5	9	10
pH 1.8 2.8 3.8 4.8 5.8 6.8 7.8	Thin layer													
	0	3.3	0	3.5	1.3	5.3	0	0		0.6	6.0	0	15.0	16.0
	8.0	0	0	0	0	8.0	30.0	0		11.5	20.0	35.0	0	0
	8.8	1.4	0	3.0	0	11.3	30.0	2.7		0	40.0	2.5	0	0
	0	10.6	0	6.0	0	0	9.0	0		8.0	2.7	35.0	13.7	0
	0	0	0	4.3	0	0	0	0		0	0	4.0	0	0
	0	0	0	0	0	1.3	0	1.0		0	0	0	0	0
	0	0	0	0	0	0	0	0		0	0.7	0	0	0
Agar 2% Suc. 20% Agar 1% Suc. 10%	Thick layer													
	0	26.7	12.5	90.0	4.0	100	100	100		3.7	100	100	25.0	100
	3.6	90.0	70.0	100	100	100	100	100		50.2	100	100	90.0	100

gelatin without buffer solution the pollen-grains germinated very well and the shifting of optimum pH during the flowering season was not seen. In the agar medium (Table 10) with buffer solution no germination was observed when the medium is alkaline, as seen in the former experiments (Table 5, 6, 7). In the earliest and in the latest stage pollen-grains germinated in a narrow range of pH; in the middle stages they germinated in a wide range of pH. The optimum pH for germination shifted from 2.8 to 4.8. In agar (1.0~2.0% agar, 5.0~10.0% sucrose) the addition of buffer solution was harmful, while in the control agar medium with sucrose but without buffer, 100% germination was observed. Harmful effect is signified by the decrease of germination ratio and

the narrowing of germination pH range (Table 10). Susceptibility to harm differs daily according to the physiological condition of pollen-grains as shown in the results obtained on different days. On the days when injury was seen in the medium with buffer solution, some injury was also seen in a highly condensed medium even though it does not contain buffer.

Discussion

Molisch and also other investigators who used liquid medium added with acid could not obtain unambiguous results. The author, however, observed 100% germination ratio in gelatin medium. The large content of gelatin hindered the elongation of pollen-tubes, but did not influence germination. The pollen-grains did not germinate at all on agar either below 1.0% or over 5.0%. The maximum germination ratio on pure agar which was seen at 4.0% was only 46%. But on agar medium the elongation of pollen-tubes was more pronounced than that on gelatin medium. When the embedding of pollen-grains in medium is shallow, they generally looked dark. The fact that the germination ratio on a thin agar layer increases by the addition of sucrose to the medium is so far considered to be caused by water supply through deliquescence of sucrose. Judging from the fact that the pollen-grains in sucrose agar medium look more transparent than those in the medium without sucrose, the former state must indicate the larger degree of embedding of grains in the medium. Pollen-grains can not directly absorb air humidity, but they germinate on behalf of water absorbed into medium from the air. A similar phenomenon was reported in the previous paper on *Cosmos* (1954). The pollen-grains do not adhere well to the surface of the agar medium, but, when it becomes warmer, the germination ratio increases on agar medium containing sucrose so that it attains to 100% in June, because of the increase of deliquescence of the medium exposed to the saturated humidity in a moist chamber. On a thick layer of gelatin, where the pollen-grains are set better, the ratio is also greater. But even on a gelatin layer, the pollen-grains do not adhere to it so good as on an agar layer, if it is thin. But at a higher temperature adherence of the pollen-grains to a thick gelatin layer is too strong and germination is considerably affected.

According to the experiment of von Berg the pollen-grains germinated only in more acidic side than pH 3.1, and NaOH hindered germination. According to the author's experiment the harmful action of ions is weaker in a thin layer than in a thick one.

By adding buffer solution one can adjust the hydrogen-ion concentration of the medium to its best for germination. By such treatment one can succeed in germinating pollen-grains in poor medium such as agar. However, even at the best hydrogen-ion concentration, the germination ratio will not be high because of the harmful effect of ions in buffer solution. The author found in gelatin the best medium, on which perfect germination was observed when the medium did not contain buffer.

Conclusion

Pollen-grains such as those of Compositae and *Rhododendron* can germinate normally only when water absorption is restricted. The favourable condition for germination of *Cosmos* has recently been explained by the author, and the germination of *Rhododendron* can also be explained similarly and more exactly.

Liquid medium is an unstable water source, and would supply water unlimitedly as long as it retains water. Such is not favourable for difficultly germinating pollen-grains. The increase of concentration of liquid medium retards water absorption of pollen-grains but does not restrict its amount, so by the addition of sucrose into the liquid germination can not be made better. Molisch found that the addition of malic acid is quite adequate for germination. Such substances may physiologically restrict water absorption of grains, when adjusted to a suitable hydrogen ion concentration, while they supply water, *i.e.* air humidity condensed by deliquescence. Nicotin recommended by von Berg restricts water absorption for germination to a considerable extent. If nicotin is added together with malic acid the former reduces the favourable action of the latter.

Colloid solution of 2.0~12.0% pure gelatin is a perfect culture medium. The favourable state be kept when a liquid layer is deep or a gel layer is thick. Besides, the optimum amount of malic acid for germination was in a deep layer of dilute solution or in a shallow layer of concentrated solution. So that germination in a moist chamber concerns the total amount of solutes in a liquid preparation.

When water deficit in medium is made up by air humidity, the hydrature of the medium increases gradually and pollen-grains germinate well. If a medium contains more than 12.5% gelatin (Manufactured in Juji Factory at Osaka), pollen-grains can not adhere to the gel surface well. But if sucrose is added, the medium works well because sucrose deliquesces in a moist chamber.

Physiologically water absorption is active in acids (pH 1.8~7.8) if it is not extremely strong, and in bases of over pH 7.8 it is inactive. The pH range removes to basic side in the most flowery season.

There are observed harmful ion actions of buffer solution, hence good result is only obtained when the amount of buffer solution is small and the layer is thin.

Agar itself is not a good material as medium, but, if it does not contain buffer, is favourable for germination, the ratio reaching 100% at a warmer temperature of June. Pollen-grains attach closely to the medium layer as the temperature rises, and at a moderate temperature they attach best. The pollen-grains obtained from flowers untimely produced outdoors in January could germinate under a warm condition but not at room temperature.

For germination the weather is too cold in May, but favourable in June. It has not yet been decided whether such temperature function is related to the chemico-physical feature of medium or the physiological feature of a pollen-grain. The physiological ability of water absorption of a grain, too, changes

daily. Decline of the ability is seen in the decrease of germination ratio and the narrowing of the germination range of pH in buffered medium and that of the amount of solute in medium.

Summary

1. Germination experiments were made with pollen-grains of a few ericaceous plants using agar and gelatin as culture media.

2. Two factors control germination: the one is the chemical feature of the medium, and the other the attaching state of pollen-grains on the surface of the medium layer.

3. In a moist chamber the attaching state of pollen-grains is controlled by the different feature of colloids which are used as medium and the deliquescence of chemicals in medium. When pollen-grains look vivid and transparent, they are regarded to be in the best attaching state. In such a state the pollen-grains absorb water just enough and not too much for their germination and pollen-tube elongation.

4. The pollen-grains germinate to 100% on gelatin without chemicals.

5. Gelatin is the best medium giving the best attaching state which is also the most favourable condition for germination.

6. Agar is a poor medium. But in a moist chamber, if chemicals are contained in the medium, they condense water sufficient enough to provide good attaching state of pollen-grains on the surface of medium layer.

7. If the layer is thick, the addition of chemicals to the medium except sugar makes it unfavourable for germination. The bad effect of chemicals seems to decrease when the layer is thin. It is due to the decrease of the effect of the chemical feature of the medium against the hydrature feature or the attaching state of the pollen-grains.

8. By adding buffer one can adjust to the best hydrogen-ion concentration for germination. By such treatment one can succeed in germinating pollen-grains on a poor medium such as agar. However, even at the best hydrogen-ion concentration the germination ratio may not be high because of the harmful effect of ions in buffer solution.

9. In an agar medium the pollen-tubes elongated well and rapidly, but germination ratio was low. Dry grains absorbed water rapidly. If water absorption was too rapid, it has a harmful effect upon germinative ability. In a gelatin medium, pollen-grains absorbed water gradually, and the germination ratio was high, although the elongation of tubes slowed down.

10. It is certain that pollen-grains do not germinate regularly in the medium with malic acid or nicotin even in June, the best time in a year. Even with the optimal amount of malic acid germination ratio did not exceed 40%.

11. If malic acid solution is used, germination in a moist chamber depends upon the total amount of the acid and not water in the medium.

12. At a higher temperature, pollen-grains germinated to 100% even on agar layers when the medium contained sucrose but not buffer, and this medium provided the best attaching state for pollen-grains, when agar became softer

and solutes deliquesced more.

13. In the earliest and latest stage of the flowering season pollen-grains germinated only in a limited range of hydrogen-ion concentration (pH 3), but the range widened in the middle stage (pH 1.8~7.8). Throughout the flowering season the optimum pH gradually extended to the alkaline side from the acidic.

14. The relation between pollen-grain germination and surrounding conditions is systematically explained.

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Intracellular Condition which Control the Interchange of Starch and Sugar in Plants

By

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A great deal of investigations have been made in regard to the formation of starch in plants in order to make clear the effect of nutrient elements on the growth of food plants (e.g. Halsall, 1948; Mitsushashi, 1948; Jacob, 1949; Hamashima, 1950; Rubin, 1950; Stanton, 1951; Sugiyama, 1953; etc.), or to make the activity and distribution of phosphorylase in the plant tissues (e.g. Day, 1937; Yin, 1947, '48; Eyster, 1949; Dyar, 1950; Gozette, 1951; Maruo, 1952, '53 etc.). There are, however, few examples of research which concerns the mechanism of starch-sugar interchange and the physiological cell conditions on the occasion of starch synthesis or breakdown in plants (Ono, 1949, '50, '51, '52, '53; Fukuda, 1952). It is clear that starch is synthesized by the action of phosphorylase *in vitro* and *in vivo* from Glucose-1-phosphate (G-1-P) as substrate (Cori *et al.* 1937; and many others), but the intracellular condition for enzymatic starch synthesis by means of phosphorylase in plant remains obscure yet.

By observing starch formation by phosphorylase in radish tuber, guard cell (Ono, 1949, '50, '51, '52, '53) and *in vitro* experiments, the intracellular condition which controls the interchange of starch and sugar in plants may be explained. In the case of radish tuber, the increase or decrease of the amount of starch in growing season and in some parts of tuber is very remarkable in some varieties, so that we can see the change of various physiological factors which accompanies the change of the amount of starch; and in the case of guard cells, the interchange of starch and sugar in each others, taking place in a short time under various physiological conditions is to be observed (Ono, 1951, '52, '53). By considering the mutual correlation of the changes of those physiological factors, the intracellular condition at the stage of the starch formation the starch-sugar interchange, and therefore the physiological and ecological meaning of these phenomena can be elucidated.

Experimental

Materials: Various varieties of radish (*Raphanus sativus* L.), especially Kôchin, Eichin (Chinese radishes), and Risô, Nerima, Miura, Miyashige, Minowase etc. (Japanese common radishes) were used and *Solanum tuberosum* L. were used mainly for the extraction of phosphorylase, *Ipomoea Batatas* Lam. for the extraction of β -amylase. Sometimes *Vicia Faba* L. was used for the preparation of phosphorylase.

Methods: The method adopted in the quantitative analysis of phosphate in

the free and esterified form as that of Allen (1940), and for the determination of α -amylase activity the method of Wohlgemuth (1908), and for reducing sugar the method of Hanes (1929), and for starch the acid resolving method were applied. Osmotic values were measured by the cryoscopic method using Beckmann's thermometer, and pH value was estimated by means of antimon electrode within pressed sap of plants. Colorimetric method was also used to determine the pH value in cells and tissues. The activity of β -amylase was determined by measuring the amount of reducing sugar in the 1% solution of soluble starch produced by its action. Total acidity was shown by the amount of 0.1 N alkali solution consumed to neutralize 10 cc. of the pressed sap of plants in the phenolphthalein indicator. The action of phosphorylase *in vitro* was determined quantitatively by measuring the amount of free phosphate liberated from G-1-P as the result of synthesis of starch, and qualitatively, the histochemical methods of Yin (1949, '50) or of Dyar (1950) were employed. The preparation of α -amylase was made by the method of Myer (1947) and that of β -amylase by the method of Balls (1948) and they were used as "crude paste". G-1-P was prepared by the method of Hanes (1940) and applied as potassium salt.

Result

A. **The existence of phosphorylase in radish tuber.** By means of histochemical methods of Yin or Dyar, the existence as well as activity of phosphorylase in the tissues of radish tuber was confirmed from the fact that starch grain is formed therein from G-1-P used as substrate (Table 1).

Table 1. The amount of starch grains formed in the tissues of radish tuber in the G-1-P solution as substrate.

Radish varieties	pH value of the tissues	The amount of starch		
		At the beginning	After 24 hrs. in G-1-P sol.	Control
Kôchin	5.2	+	†	+
Eichin	5.0	†	††	†
Risô	5.2	+	†	+
Nerima	5.4	—	+	±
Minowase	5.2	—	†	—
Miura	5.4	—	+	+

The phosphorylase extracted from radish tuber by means of Hanes' method (1940), synthesize starch from G-1-P as substrate and liberate free phosphate from the substrate as potato phosphorylase did. (Table 2).

B. **Seasonal change of the amount of starch and other physiological factors in growing radish tuber.** The results obtained are shown in Fig. 1. It is apparent from this figure that the change of the amount of starch can be divided into two period, the period of starch forming and that of consuming.

Table 2. The amount of free phosphate liberated from 10 cc. 0.01 N. G-1-P sol. by the action of phosphorylase extracted from potato and radish tuber.

	Tempera- ture °C.	pH value	Amount of phosphate (mgr.)						
			Time (hr.) of incubation					Increase of free phosphate	Total phos- phate
			0	1	2	3	4		
Potato phosphorylase	30	6.0	0.15	0.53	1.52	1.55	1.72	1.57	3.24
Radish phosphorylase	30	6.0	0.15	0.24	0.38	0.53	0.72	0.57	3.24

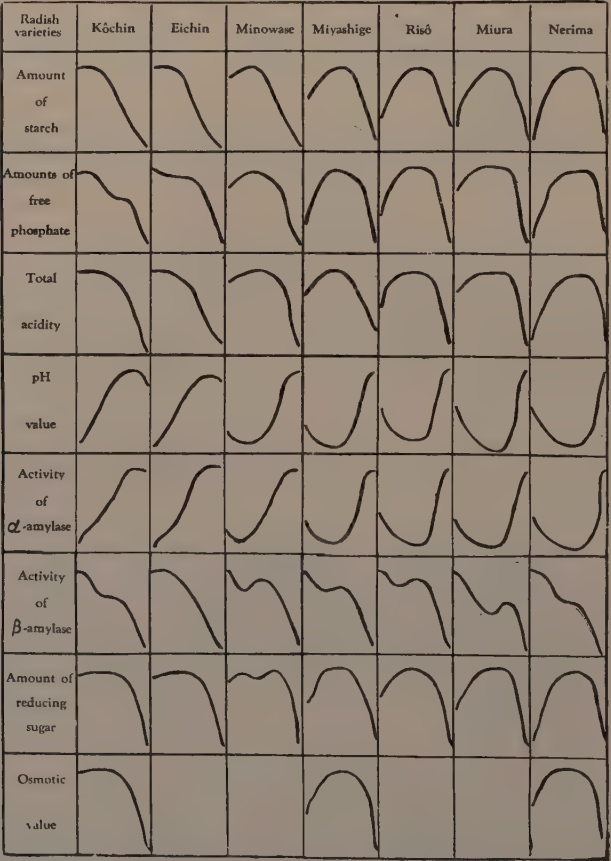


Fig. 1. The seasonal changes of physiological factors in tubers of radish varieties.

In Kôchin and Eichin (Chinese radish varieties) the amount of starch attains its maximum in the early period of growth, and then gradually decreases toward the later period. But in Minowase or in Nerima, it attains its maximum in the middle or later period of growth and in the other varieties such as Miyashige,

Risô and Miura, between the two. According to the starch formation or consumption the curves which show the amount of free phosphate and that of reducing sugars, osmotic value or total acidity are parallel with starch, but the curves which show the activity of α -amylase and pH value are inverse to the change of starch in various growth periods. The activity of β -amylase becomes weaker from early toward late growth period, but it temporarily becomes more intense at the turning point of starch forming and consuming period. By the length of starch forming period in radish tuber, radishes can be

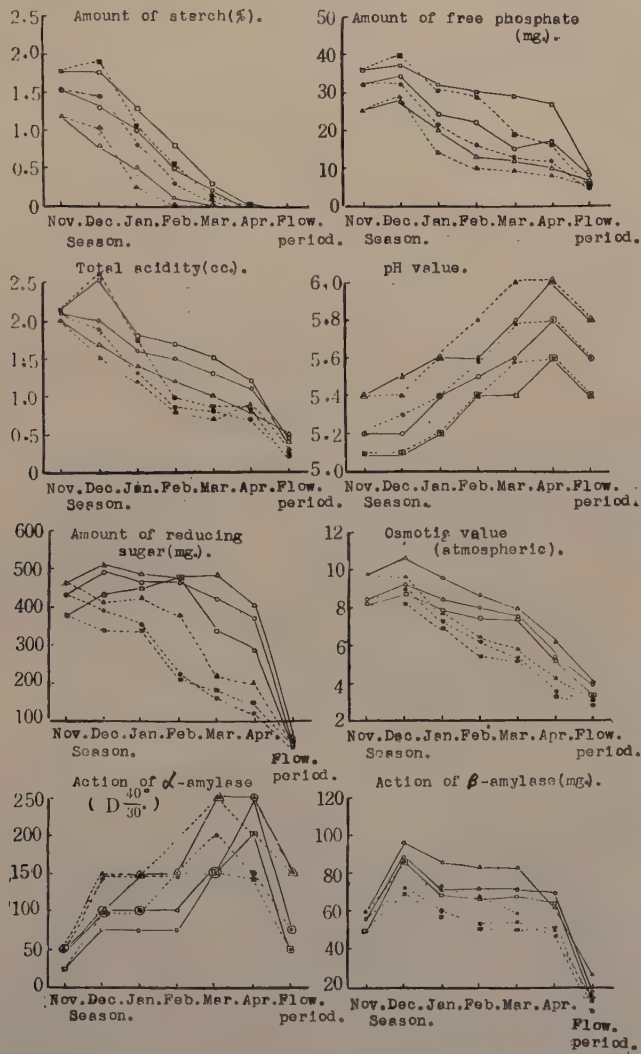


Fig. 2. (A). Kôchin.

Fig. 2. The seasonal changes of physiological factors of upper (△ mark), middle (○ mark) and lower (□ mark) parts of radish tubers in normal conditions (full line) and in long day treatment (dotted line).

divided into three types, early, middle or late types.

Mutual relation or reciprocal action of various physiological factors seems also to be very close, and it is marked particularly between the total acidity and the pH value, between the amount of reducing sugar and the osmotic value,

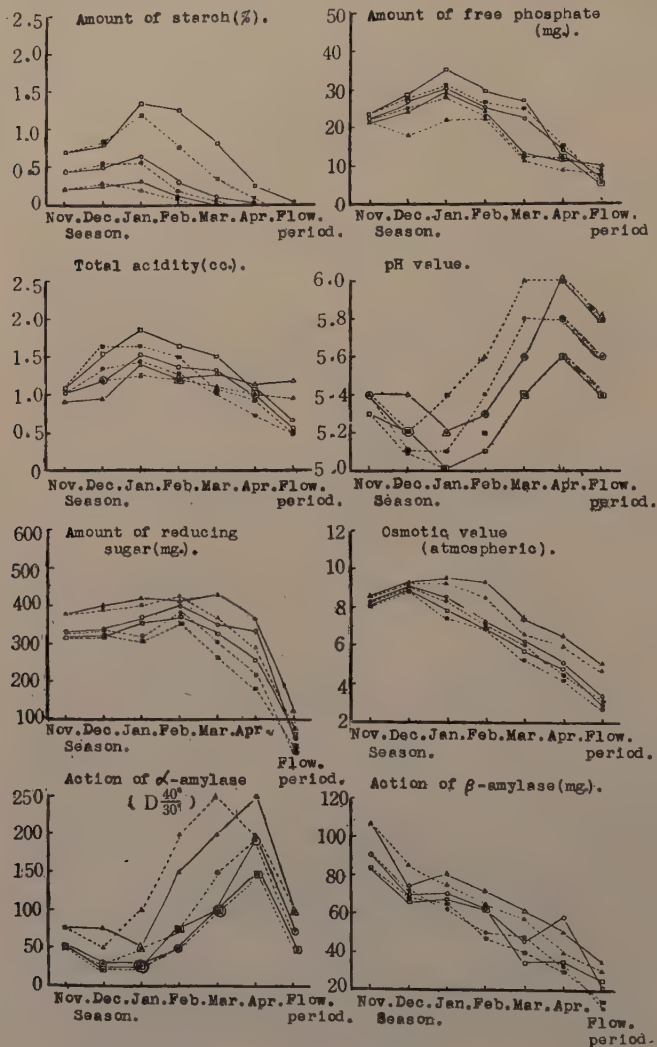


Fig. 2. (B). Miyashige.

between the pH value and the amount of free phosphate or the action of phosphorylase or amylase, between the action of amylase and the amount of reducing sugar and between the amount of starch and the free phosphate or reducing sugar.

C. The changes of the amount of starch and other physiological factors in various parts of radish tuber. A radish tuber is divided into three parts:

the upper, middle and lower part, and the seasonal change of various physiological factors observed in them are shown in Fig. 2.

1) **Starch:** Starch is formed first in the lower part, then in the middle part and finally in the upper part of radish tuber and the consumption of starch takes place in reverse order, so that the amount of starch is always more in

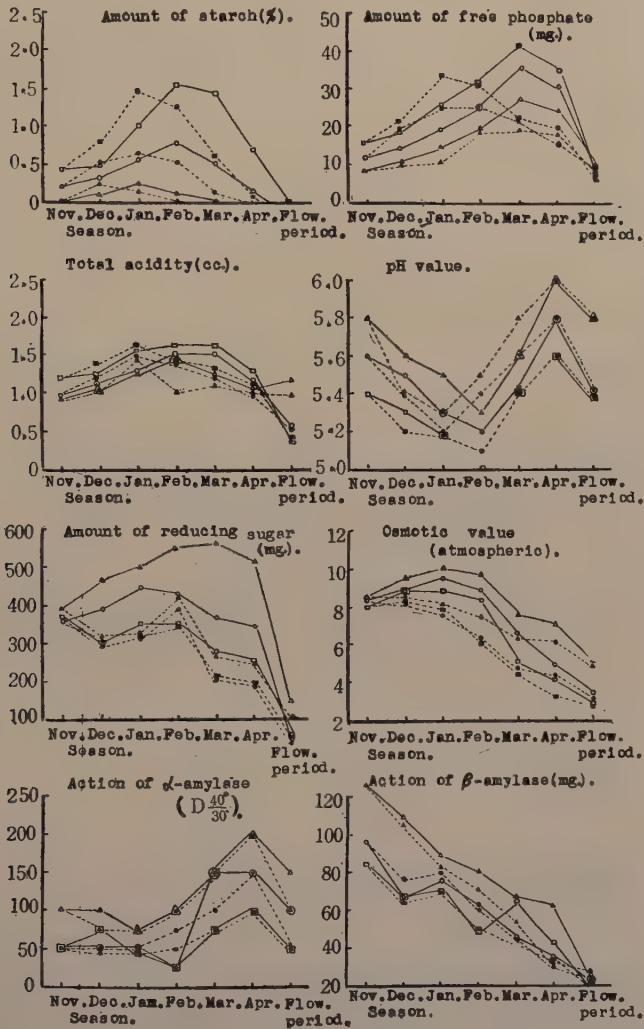


Fig. 2. (C). Nerima.

the lower part than in the middle or the upper. For these reasons, the amount of starch in the upper part attains its maximum in the early period of growth and also the consumption goes faster than in the lower part. For instance, in Nerima, the amount of starch in the upper part attains its maximum in January

and then decreases fast, but in the middle and the lower part, the maximum is attained in February and decreases more slowly especially in the lower part, which contains comparatively much starch even in April when it was consumed in the upper and middle part.

2) **Reducing sugar and osmotic value:** The upper part contains more reducing sugars than the middle or lower unlike the case of starch, and from the upper toward lower the reducing sugars become less in quantity. The change of osmotic value is parallel with the change of the amount of reducing sugar, i.e. in the upper part it is higher than in the lower part, and especially in cold winter season, from December to February, it is higher and shows 8~9 atmospheric pressure, but in April when in bloom, it suddenly comes down and shows only 3~4 atmospheric pressure. It is a noteworthy feature that these two factors change without showing any relation to seasonal change of the amount of starch and show very high amount and value in the upper part, especially, in the cold winter season; for instance, even in Kôchin, the early varietal type, without reference to the remarkable decrease of starch from December to February, the reducing sugars do not decrease so much in that season.

3) **pH value:** pH value is larger in the upper than in the lower part and becomes smaller from the upper toward the lower. In the starch forming period it is as small as 5.0~5.4, but in the starch consuming period, it is as large as 5.6~6.0. The curve which shows the change of the pH value is completely reverse as compared with that which shows the starch content, and remarkably goes large when in bloom, but in later period of growth it goes small again to be 5.4~5.6

Table 3. The seasonal change of pH value and the amount of starch (%) in the upper, middle and lower part of a radish tuber, variety Risô.

	Part	Jan. 10	Jan. 20	Feb. 1	Feb. 10	Feb. 20	Mar. 1
Starch (%)	Upper	0.48	0.54	0.55	0.57	0.48	0.32
	Middle	0.76	0.96	0.98	1.02	1.04	0.65
	Lower	1.15	1.25	1.39	1.45	1.69	1.25
pH value	Upper	5.5	5.4	5.2	5.6	5.3	5.6
	Middle	5.4	5.4	5.3	5.2	5.4	5.2
	Lower	5.1	5.1	5.0	5.0	5.4	5.2

In this remarkable periodical change of pH value due to growth, especially in turning point from starch forming to consuming period, more complex change is to be seen (Table 3). For example, the pH value suddenly grows larger just at the time when the amount of starch attains its maximum and consumption thereof takes place, but after that it again suddenly becomes smaller and then gradually becomes larger with the consumption of starch.

The phenomena observed in radish tubers were also seen in various growing period of potato tuber or of cotyledon of bean seed (*Vicia Faba* L.) as shown in

Table 4. The pH value in mature state is comparatively larger, but it then suddenly goes small and gradually grows larger when sprouting begins.

4) **Total acidity:** Total acidity is larger in the lower than in the upper part except in flowering period, and is gradually decreases from the lower toward

Table 4. The change of pH value in various growing periods of potato tuber and cotyledon of bean.

Materials	pH (Growing period)				pH (Resting period)	pH (Sprouting period)
	Early	Middle	Late	Mature		
<i>Solanum tuberosum</i>	5.0-5.2	5.4	5.6-5.8	6.0-6.2	5.0-5.4	6.0-6.4
<i>Vicia Faba</i>	5.2	5.4-5.6	5.8	6.0-6.4	5.8	5.4-6.0

the upper part. This change is completely parallel with the change of the amount of starch and that of hydrogen ion concentration (reverse when shown by pH value) in every growing period or part of a radish tuber. The change of total acidity in a potato tuber is shown in Table 5. In this table we can see the remarkable change of total acidity even in the resting period and the gradual decrease of it toward the sprouting period.

Table 5. The seasonal change of total acidity, content in reducing sugars and activity of α - and β -amylase in the potato tuber.

Season	November	December	January	February	March, sprouting	April, after sprouting
Total acidity cc. N/NaOH	8.4	7.2	6.0	5.1	3.8	1.7
Activity of α -amylase ($D_{40}^{20}/30'$)	10	20	25	30	45	45
Activity of β -amylase (mg. of red. sugars)	8.48	10.14	22.78	29.18	33.12	41.40
Reducing sugars mg. in 10 cc. pressed juice)	57.88	103.50	160.46	252.54	289.80	389.36

5) **α -amylase:** The action of α -amylase is always stronger in the upper part than in the lower and is weaker from the upper toward the lower part in starch forming period. In an individual, however, consuming period the activity grows remarkably strong, and when accumulated starch is almost consumed, the activity of α -amylase falls again. There is a close relation between the activity of α -amylase and pH value.

6) **β -amylase:** As in the case of α -amylase, the activity of β -amylase is stronger in the upper than in the lower part, but little close relation exists between it and the seasonal change of the amount of starch, that is, in starch

forming period the action of β -amylase becomes weaker and even in starch consuming period it does not become stronger as that of α -amylase.

7) **Phosphate:** The amount of free phosphate is greater in the lower than in the upper part of a radish tuber and changes in parallel with the change of starch contents in starch forming period, but in starch consuming period the decreasing rate of free phosphate is not so pronounced as compared with starch content. In flowering period the decrease of free phosphate is, however, remarkable. These relations are shown in Table 6 and Fig. 3.

Table 6. The increase and decrease ratio of free phosphate and starch contents in different growing periods of a radish tuber.

Season		December	January	February	March	Early April	April (flowering)
Kôchin	Starch	+13.3	-30.7	-48.9	-63.04	-96.5	-98.5
	Free phosphate	+ 4.5	-22.9	-14.3	- 8.9	- 7.7	-49.5
Eichin	Starch	+13.07	-26.1	-55.8	-61.6	-83.9	-90.4
	Free phosphate	- 5.7	-35.9	-26.6	- 5.7	- 6.5	-52.5
Risô	Starch	+30.4	+38.3	+15.6	-41.6	-51.9	-98.5
	Free phosphate	+14.4	+29.7	-19.5	- 8.9	- 6.5	-45.1
Mino-wase	Starch	+30.3	-23.2	-30.3	-30.3	-56.2	-82.9
	Free phosphate	+ 5.4	+ 8.7	- 9.05	-19.9	- 8.2	-41.2
Miura	Starch	+19.4	+39.5	+ 5.0	-26.9	-55.8	-98.2
	Free phosphate	+15.7	+ 4.3	+27.9	- 1.8	- 3.9	-52.4
Nerima	Starch	+42.8	+86.6	+42.8	-18.7	-53.8	-95.2
	Free phosphate	+21.5	+40.4	+25.6	+39.6	-14.8	-65.8

When radish was cultured in Knop's solution which contained various concentrations of potassium phosphate, the higher the concentration of it in the medium was, the more the free phosphate was contained in the radish tuber, and in these cases, when the content of potassium phosphate in Knop's solution was made to 5%, the formation of starch was checked, but from 0.1 to 1% the formation of starch was accelerated. These results are shown in Table 7.

Table 7. The contents of starch and phosphate in a radish tuber cultured in Knop's solution, containing potassium phosphate of various concentrations.

KH_2PO_4 (%)	Starch (%)	Free phosphate (mg.) per 100 cc. pressed juice	1-ester phosphate (mg.) per 100 cc. pressed juice
0.1	0.14	18.56	13.88
0.5	0.18	30.79	15.98
1.0	0.25	31.22	21.21
5.0	0.20	45.45	15.32
0.0	0.15	15.44	12.41

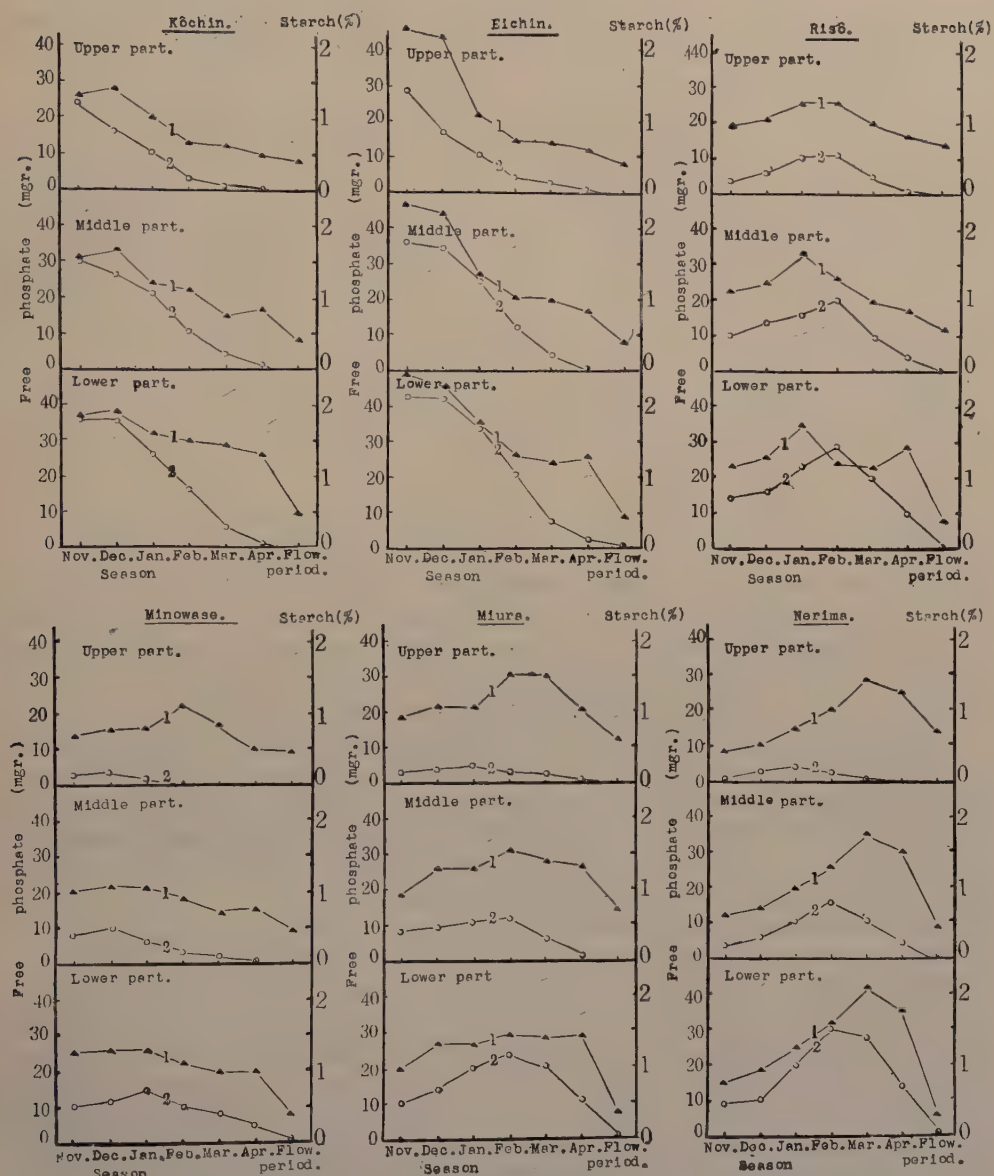


Fig. 3. The seasonal changes of the content of free phosphate (1 or ▲ mark) and starch (2 or ○ mark) in three parts of tuber of various radishes.

As shown in Table 8, the ratio of free phosphate/G-1-P in various radishes in growing period from December to February are very changeable with the change of starch content, especially by the change of starch formation or consumption period. In Kôchin and Eichin which are shown in starch consumption period, the ratio decreases from 4.8 to 2.9, accompanying the starch decrease.

But in Risô, Nerima etc. which are in starch formation period the ratio increases from December to February.

Table 8. The seasonal change of starch contents and the equilibrium constant (free phosphate/G-1-P) in a radish tuber.

Varieties	December		January		February	
	Starch (%)	Equilibrium constant (free p./G-1-P)	Starch (%)	Equilibrium constant (free p./G-1-P)	Starch (%)	Equilibrium constant (free p./G-1-P)
Kôchin	1.3	4.8	0.9	4.8	0.46	2.9
Eichin	1.53	7.9	1.13	6.1	0.6	3.5
Risô	0.6	3.9	0.83	5.0	0.96	5.0
Minowase	0.43	2.3	0.33	3.4	0.23	4.9
Miura	0.42	2.8	0.6	3.7	0.63	5.6
Nerima	0.3	1.9	0.8	3.07	0.85	5.02
Hôryô	0.5	3.7	0.7	5.2	0.2	2.5

D. The change of various physiological factors from the upper toward the lower part of a radish tuber in various growing periods. The change of various physiological factors in six periods, which was observed in eight parts of equal length of a radish tuber is shown in Fig. 4. There are also intimate relations between mutual factors as in the seasonal change (Fig. 2). For instance, the amount of starch gradually increases from the upper toward the lower parts, the total acidity and free phosphate contents rise up in parallel from the upper toward the lower parts, while, on the contrary, the activity of amylase and pH value go down from the upper toward the lower parts. The amount of reducing sugars goes down from the upper toward the lower parts unlike the amount of starch.

E. The effect of pH value on the activity of amylase and phosphorylase.

Results were shown in Fig. 5. The activity of α -amylase, in general, was stronger in large pH values and the optimum lay at pH 7.0 or thereabout, while on the other hand, the activity of β -amylase was strong in small pH values and optimum action existed in pH value from 4.0 to 5.0. The activity of phosphorylase was strong in pH values from 5.0 to 6.0, and the optimum pH value lay between 5.0 and 5.6 in the case of α - and β -amylase activity. In general the activity of β -amylase was strong in a comparatively wider range of pH values than α -amylase and the phosphorylase activity which was confined within narrow range of pH values.

F. The effects of long day treatment on the change of various physiological factors in a radish tuber. As shown in Fig. 2, the seasonal change of various physiological factors in plants, subjected to long day treatment proceeded 1–1½ months earlier than in those grown under normal conditions. Especially in Miyashige, a middle type, the content of starch attains its maximum in December, seemingly transforming into an early type, and in Nerima, a late type, in December in the upper part and in January in the middle part, so that this

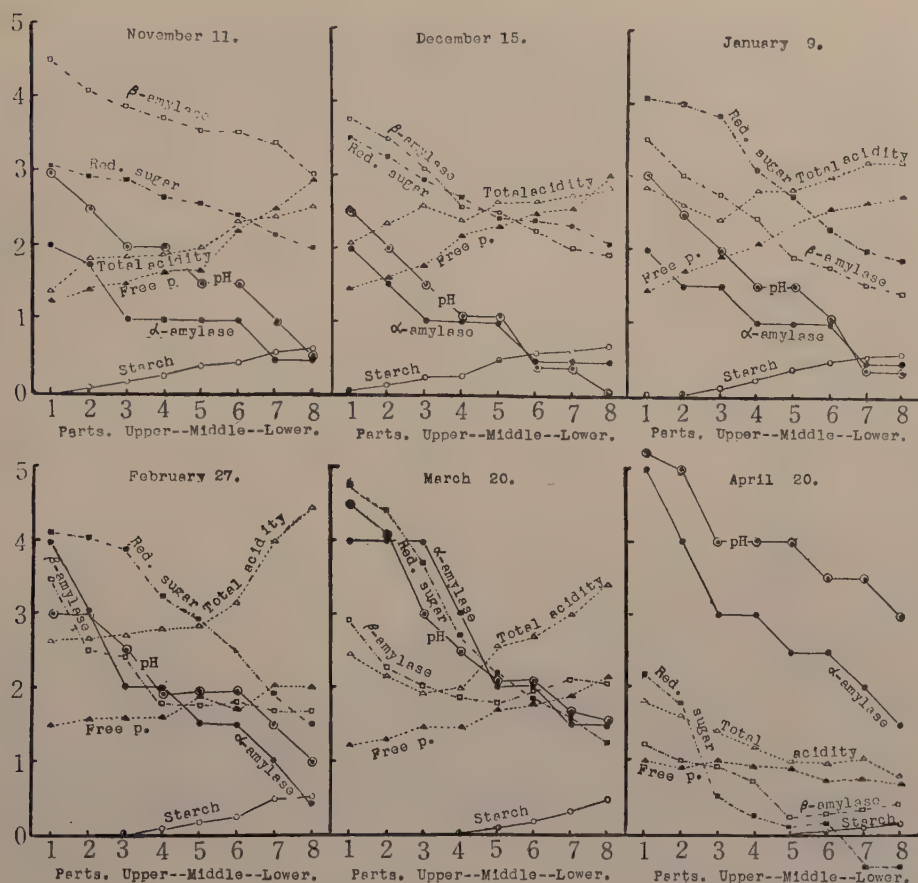


Fig. 4. Periodical change of physiological factors in eight equal parts of radish tubers. The order of parts is arranged in abscissa from upper to lower. The ordinate: 0~5 means pH value (5.0~6.0), total acidity (0~2.5 cc.), contents of free phosphate in 100 cc. pressed sap (0~50 mgr.), amount of starch (0~5%), amount of reducing sugar (100~600 mgr. in 10 cc. pressed sap), activity of α -amylase (0~250 $D_{40^\circ}/30'$), and the activity of β -amylase (20~120 mgr. reducing sugar in 10 cc. soluble starch).

variety apparently changes into a middle type. With this change of starch content, the various other physiological factors and the mutual relations between them also change as explained above. The content of reducing sugars seems to be comparatively smaller and might have been effected by the intensive heat evoked by an electric light used in long day treatment.

Discussion

When we consider the interchange of starch and sugar in plant cells, not only the reversible action of phosphorylase but also the action of amylase must

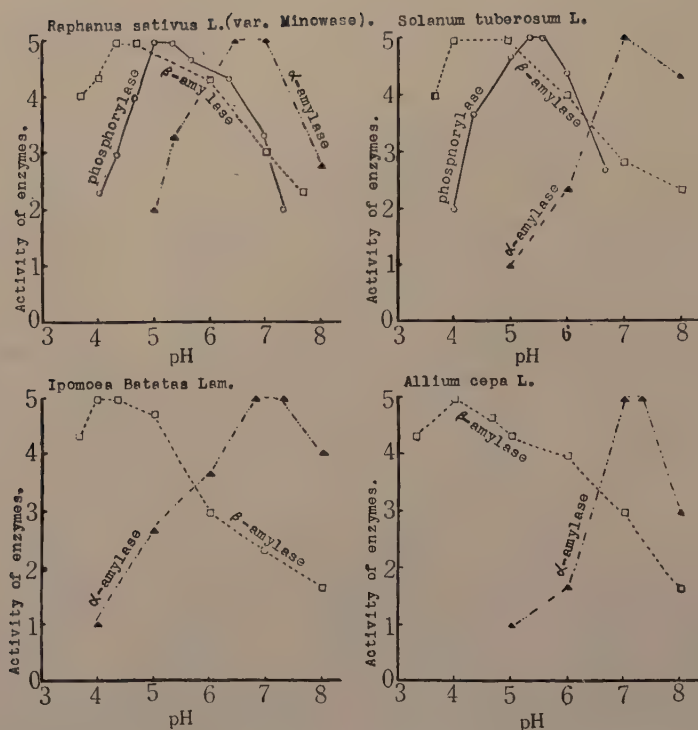


Fig. 5. The action of amylase and phosphorylase in different hydrogen ion concentrations in various radish plants. The action of these enzymes is shown on the scale 0~5.

be taken into account.

A. **The relation of starch synthesis to the change of reducing sugar contents and the osmotic value.** Sugar is not only needed as nutrient for plant growth but also plays an important part of adaptive osmoregulation to the change of natural conditions (Yoshii, 1935; Jimbo, 1936; Ishibe, 1936; Fukuda, 1952; et al.). The increase of sugar content promotes the synthesis of starch in shoot and root in normal conditions. But in the winter season, meteorological factors, especially lower temperature, control the content of sugar or osmotic value in shoot and also in the upper part of root (Fukuda, 1952). This phenomenon was observed in a radish tuber. For instance, in the upper part of the tuber more reducing sugars are contained in spite of a small amount of starch than in the lower part, especially in winter extending from December to February, but in flowering period it decreases suddenly. In parallel with this change of the amount of reducing sugars, the osmotic value changes and becomes high in cold winter in the upper part. Thus a radish tuber adapts itself to cold winter. After all in a radish tuber, the contents of starch and sugars, and osmotic value change in parallel with each other according to season (Fig. 2), but in some parts, this paralleism is broken and becomes even reverse (Fig. 4). For this fact the

meteorological factors might be responsible.

B. The effect of pH value on the change of various factors. 1) The relations between the change of starch content and pH value and total acidity:

The relations between the change of starch content, pH value, and total acidity are so close that the change of starch content and starch forming or consuming period could be demonstrated by successively estimating the change of pH value or total acidity according to seasons or in various parts of a radish tuber. Namely, the content of starch is much in the lower part of a radish tuber where pH value is small, and the total acidity is large, and this relation is reverse in the upper part. In starch forming period with the increase of starch content and total acidity the pH value becomes gradually smaller and in starch consuming period the relation is completely reverse. These two sets of relations are the same with those observed in an individual radish tuber from the upper toward lower parts and from the lower toward the upper parts, and the former is similar to the relation observed in starch forming period and the latter to those in starch consuming period. Other plants such as potato and bean also show similar phenomena.

In guard cells, starch synthesis is more active in smaller pH values but less in larger pH values (Ono, 1951, '52, '53). And the change of pH values is controlled by the change of temperature and light intensity. For instance in guard cells treated in dark or at higher temperatures the pH value is smaller than that in guard cells treated in light or at lower temperatures. In this condition of small pH values, the synthesis of starch in guard cells is more active than in the condition of larger pH values (Ono, 1952, '53).

2) The relation between the change of pH value and the action of enzyme:

As mentioned above, the change of pH values is controlled by the change of temperature or light, and has an intimate relation to the formation of starch, but on the other hand the change of pH values chiefly controls the activity of enzymes in living cells. The optimum pH value for phosphorylase is 5.0~5.6 in radish, and generally 5.0~6.0 in Irish potato and sweet potato (Nakamura, 1952; Arreguin, 1949; Hopkins, 1953; Kerred, 1951). In the starch forming period of a radish tuber, the pH value for β -amylase is larger than that of phosphorylase and for α -amylase, smaller as is shown in Fig. 5. Also in other plants, the pH value in the cell between 5.0~6.0 is generally believed to be good for the action of phosphorylase and formation of starch. However in starch consuming period, the gradual increase of pH value is convenient for α -amylase but not for phosphorylase and β -amylase.

When these enzymes act independently *in vitro*, they are active in a comparatively wider range of pH values, but when these enzymes act side by side as is generally the case with the cell, they seem to be active only near their own optimum pH values. This phenomenon is clearly to be seen in the change of the contents of starch, pH values and the activity of amylases in a radish tuber as experienced in different seasons and individuated parts of tuber.

3) The relation between the change of pH value and the phosphate content:

If starch is synthesized from G-1-P or decomposes to G-1-P by phosphorylase, the increase or decrease of starch content might change with phosphate in

parallel (Myrbäck, 1949; Hassid, 1951; et al.). In the case of radish tuber, this phenomenon was confirmed in the starch forming or early consuming period. But in the middle or later part of starch consuming period free phosphate does not decrease so remarkably as starch. This shows that starch was decomposed by amylase which needs no free phosphate. On the other hand, the pH value in this period becomes gradually larger, the action of amylase being promoted and that of phosphorylase directly depressed and consequently the accumulation of sugar takes place. After the flowering period, the sugar thus accumulated, free phosphate suddenly decreased and phosphorylated into G-6-P to be consumed for nutrition of flower and for seed formation.

Phosphate has close correlation to carbohydrate metabolism in plants (Rubin, 1950). Adequate supply or absorption of phosphate promotes the synthesis of starch in plants (Ono, 1952, '53; Komatsu, 1951), but the existence of phosphate in excess checks the formation of starch. Also in the case of guard cells, the formation of starch is less pronounced when they were treated in phosphate buffer solution than in acetate buffer solution though the pH value remained the same (Ono, 1952, '53).

These phenomena can also be explained by noticing the relation between the change of pH values and phosphate contents. In general, the equilibrium constant (free phosphate/G-1-P) in phosphorylase activity depends upon the change of pH values. When pH is higher than 7.5, the constant is 2.2 and, in the case of potato phosphorylase, when pH is 5.0 the constant is 10.8, but when pH is 7.0 the constant becomes 3.3 (Sutherland, et al. 1941). In general, according that pH value is small, the equilibrium constant becomes larger and vice versa. In a radish tuber, as a result of starch synthesis from G-1-P the accumulation of free phosphate takes place so that the equilibrium constant grows larger with it, and pH value becomes smaller as to maintain the equilibrium constant. In this condition only the starch and sugar interchange declines in favour of starch formation. This can be seen in starch forming period. If free phosphate accumulates to a large extent as the result of starch synthesis from G-1-P, and pH value does not become smaller to maintain the resulted large equilibrium constant, the decrease of free phosphate caused by phosphorolysis of starch or relative increase of G-1-P must occur. Consequently in these cases the starch and sugar interchange declines in favour of sugar and of starch decomposition. All these facts are to be observed early in the starch consuming period in the case of radish tuber. When the pH value grows larger afterwards, the enzyme concerned, phosphorylase gives place to amylase, and this in turn probably to hexokinase, so that in this period the equilibrium constant (free phosphate/G-1-P) does not relate itself to the change from starch formation to consumption.

After all, the change of pH value controls equilibrium constant and also the equilibrium constant, and pH value controls the activity of phosphorylase.

C. The effect of meteorological factors. Meteorological factors, particularly long day treatment or temperature, influenced physiological properties of radish. For instance, long day treatment hastens flower formation 1~1½ months earlier than in normal condition, and the middle variety is thereby changed apparently to the early one and the late variety to the middle. Such is also the case with

different parts, i.e. the upper, middle and lower part of a radish tuber. The upper, middle and lower part of a radish tuber shows the change similar to that of the early, middle and late type, respectively. But in the upper part the influence is more conspicuous than in lower part, as is shown in low temperature. The change of physiological properties due to the change of factors in the upper part of a radish tuber is generally similar to the change in a root and stem of pine tree (Fukuda, 1952). In the upper part of radish tuber, starch is changed into sugar from December to February, causing comparatively higher osmotic value, so that the plant may adapt itself to cold winter. The content of starch and reducing sugars change parallel with each other, but, when we see from the upper to the lower part or vice versa, the relation between the contents of starch and reducing sugars is completely reverse to what takes place in the seasonal change. It is likely that the growth periodical change is controlled, especially in the upper part, by meteorological factors, because the variety Kôchin, which has a short tuber whose greater portion is exposed above the ground, does not show a greater difference in the amount of reducing sugars from the upper toward the lower part, while the variety Nerima which has a long tuber, the greater portion of which is under the ground, shows comparatively much difference of it.

Conclusion

The change of starch into sugar and vice versa which takes place in the cells of radish tuber is a physiological phenomenon needed for the autonutrition and osmoregulation, and this is the result of the synthetic activities of intracellular factors which adapt themselves to the change of external factors. As factors which control the interchange of starch and sugar, we may consider factors involved in growth and meteorological factors.

In the radish tuber, the change of starch contents can be divided into two periods, the starch forming and starch consuming. By the length of the normal starch forming period, the radish can be divided into three types: the early variety, starch content of which attains its maximum in the early period of growth, and the same is true for the middle and the late variety.

Besides the change of starch content due to growth, we can see the complex change of various physiological factors. The pH value and the activity of amylase in the starch forming stage become smaller and weaker, and the contents of reducing sugars, and free phosphate, total acidity, and osmotic value grow larger, while all these factors show reverse tendency in the starch consuming stage. The change due to growth and seasons is accompanied by the change of these factors from the upper toward the lower part and vice versa, of a radish tuber. Generally speaking, the various physiological factors in the upper, middle, and lower part of a radish tuber change in a similar manner as in the early, middle, and late variety type, respectively. By long day treatment changes the physiological factors due to growth and seasons are hastened by 1~1½ months as compared with untreated plants.

The interchange of starch and sugar is controlled by the change of pH value,

which is in turn controlled by the change of light or temperature. The change of pH values also controls the action of enzyme such as phosphorylase and amylase. The enzymes are active in a wide range of pH values *in vitro*, but around its optimum pH value, the range of pH values is small *in vivo*. The optimum pH value for β -amylase is from 4.0 to 5.0, for phosphorylase, from 5.0 to 6.0 and for α -amylase from 6.0 to 7.0. Therefore the change of pH value in the cell determines the enzyme mainly acting. In a radish tuber, in starch forming period, the optimum pH for phosphorylase is from 5.0 to 6.0. But in the starch consuming period the pH value grows larger and promotes activity of amylase.

The equilibrium constant (free phosphate/G-1-P) is determined according to the change of pH value, and when the pH value is large, it becomes smaller, and vice versa. In a condition where free phosphate is much accumulated in the cell by the starch synthesis from G-1-P or by the absorption as nutrient, but G-1-P does not increase, the equilibrium constant becomes larger and is maintained only at a small pH value. So that only at small pH value, the synthesis of starch occurs and starch is more synthesized than is hydrolyzed at a large pH value, phosphorolysis of starch and the increase of G-1-P or decrease of free phosphate must take place if the equilibrium is required to be at a small and constant level. In this condition the formation of starch does not occur. In the radish tuber, the pH value in the starch forming period is smaller than in the starch consuming.

The relation between the change of pH value and total acidity is very close and also related with the change of starch content. By measuring these factors successively, the turning point from the starch forming to the consuming period is to be known. The contents of starch, free phosphate, and total acidity become larger from the upper toward the lower part in a radish tuber, but the activity of amylase, osmotic value, and content of reducing sugars are very intimately related, and in the upper part they are larger than in the middle or the lower part and especially in cold winter days from December to February. These show that the effects of meteorological factors, especially of lower temperature on the change due to growth, and that the plant adapt itself to the cold winter through osmoregulation. Thus in the lower part of a radish tuber, the change of various physiological factors is influenced only by the periodic change due to growth, but in the upper part, this meteorological factor acts so strongly that the change of these physiological factors in the upper part seems more complex.

Summary

1. The periodical change of starch due to growth of a radish tuber can be divided into two periods: starch forming and starch consuming. Radish can be divided into three varietal types by the length of starch forming period: the early, middle and late ones. The early variety has a short starch forming period and the content of starch attains its maximum in the early period of growth, while in the late variety the circumstances is quite reverse. The middle one lies between these two.

2. With the seasonal change of starch forming and starch consuming stage,

we can see the changes of various physiological factors are intimately related. In the starch forming period, the content of free phosphate and reducing sugars, total acidity, and osmotic value increase, but the activity of amylase and pH value decrease. In the starch consuming period, the matter is reverse.

3. The content of starch, free phosphate, and total acidity grow larger from the upper toward the lower part of a radish tuber, but the activity of amylase, pH value, content of reducing sugars, and osmotic value grow smaller.

4. Starch is formed increasingly from the lower toward the upper part of a tuber and then decreases from the upper toward the lower part. In these changes, as similarly seen in the seasonal change of growth period, various physiological factors are related to the change of starch content. The change from the upper toward the lower is similar to the seasonal change of starch forming period, and that from the lower to the upper is similar to that of starch consuming period.

5. The changes of physiological factors in radish tuber can be divided into three: those in the upper part are regarded as similar to those in the early variety, and those in the middle part, to those of the middle variety, and those of the lower part to those of the late variety.

6. In starch forming period the contents of starch and free phosphate change in parallel, but in starch consuming period, the free phosphate does not decrease as starch. It is clear that in the former period, starch is synthesized from G-1-P at small pH value by the activity of phosphorylase, but in later period starch is hydrolyzed by amylase at larger pH values.

7. The change of pH value directly determines the mainly acting enzyme and brings about the transition from phosphorylase to amylase and vice versa, and it determines the equilibrium constant (free phosphate/G-1-P) in the cell. For this reason the interchange of starch and sugar is controlled by the change of pH value; for instance, the small pH value accelerates the formation of starch.

8. The accumulation or absorption from outside of free phosphate checks the formation of starch in the cell, when pH value does not become small as to maintain the adequate equilibrium constant (free phosphate/G-1-P).

9. By long day treatment, the change of the amount of starch and the physiological factors in a radish tuber due to growth and seasons occurs 1~1½ months earlier than in untreated plant. Therefore the middle variety type changes into the early variety and the later variety into the middle type.

10. Meteorological factors, especially lower temperature, disturb the change of physiological factors concerning the change due to growth of a radish tuber more in the upper part than in the lower part, so that the content of reducing sugars and osmotic value are higher in cold winter season in the upper part than in the lower.

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Some Aspects of Rhythmicity of the Protoplasmic Streaming in the Myxomycete Plasmodium*

By

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One of the characteristic features of protoplasmic streaming in the myxomycete plasmodium is the rhythmic reversal of the direction of flow. The protoplasm flows back and forth, reversal of the direction occurring usually around every one minute. This is a phenomenon striking enough to arouse the curiosity of its observers and incite them to look for its causes. For all that the mechanism of the reversal still remains a riddle.

The implicit assumption so far made by some early workers (Hilton 1908; Watanabe, Kodati and Kinoshita 1937) is that the new conditions brought about by the streaming in either one direction, such as the inner pressure which might increase at one end of the plasmodium, or any other possible physical or chemical changes accompanying the streaming, would induce the protoplasm to flow in the opposite direction. In other words, such a view is based on the premise that the streaming is a *sine qua non* element in the chain of cause and effect governing the periodic back and forth movement. According to this view, therefore, if we could restrain the protoplasmic movement in some way or other, the motive force of the flow would not be able to advance from the phase where it stands at the time.

It has, however, been demonstrated by Kamiya (1942, 1943) who stopped the protoplasmic flow continuously by applying balancing counter-pressure that, contrary to the above conjecture, the periodic generation of the motive force persists even during the standstill of the movement. This shows that the migration of the protoplasm does not play a direct part in causing the reversal of the streaming direction. There have been thus discrepancies between the view held by early workers and the conclusion derived by Kamiya.

The present work has been conducted with the purpose of giving a decisive solution to the problem. It deals with the motive force of the streaming and electrical activities of the protoplasm, the flow of which is arrested by temporal gelation. For the experiment the slime mould *Physarum polycephalum* served as material. The experiment was performed in the following manner.

Method of Gelation

There are various agents which gelate or 'set' the protoplasm reversibly. Among them, the most satisfactory agent with the least ill effect on the slime

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mould so far known being carbon dioxide (Seifriz, 1941, 1942), this gas was exclusively used for the present experiment in which protoplasm was gelled in a reversible fashion. A short while after applying carbon dioxide gas diluted with air in proper ratio, streaming stops suddenly and completely with little fore-warning. Complete recovery takes place when carbon dioxide is replaced with fresh air. When the concentration is as low as 10%, setting takes place only for a brief period, if it does at all, the flow resuming spontaneously even in the same gas mixture.

Outline of the Technique for Measuring the Motive Force

The double-chamber method developed by Kamiya (1940, 1942, 1943, 1953) enables one to oppose the force which moves the protoplasm. Two protoplasmic masses connected with a single strand is so placed in a double-chamber shown

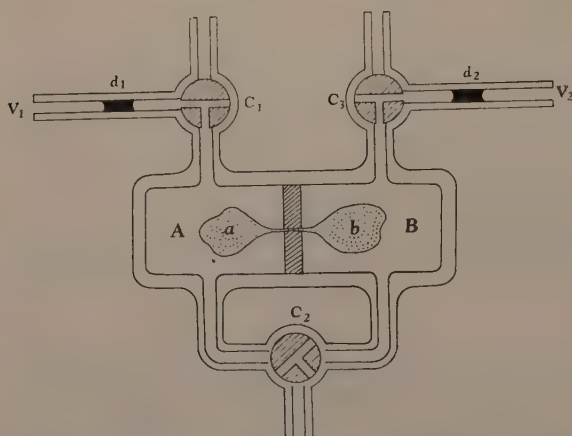


Fig. 1. Double-chamber for measuring the motive force of the protoplasmic streaming while admitting carbon dioxide gas. For explanation see text.

in Fig. 1 that protoplasmic masses *a* and *b* are placed in the different compartments *A* and *B* while the thin strand connecting them penetrates the central septum of the chamber. Air pressure in *B* may be made either higher or lower than the atmospheric pressure while compartment *A* is kept at the level of the atmospheric pressure. If there is no difference in the air pressure between compartment *A* and *B*, rhythmic back and forth streaming of protoplasm along the connecting strand goes on normally. Once

pressure is established between the two compartments, however, the flow along the connecting strand is strikingly affected. It is either accelerated or retarded, or reversed according to the direction and magnitude of the pressure difference induced. By making the air pressure in compartment *B* either higher or lower than that in compartment *A*, it is also possible to oppose the motive force in such a way as to hold the protoplasm at a standstill. The counter pressure which is just sufficient to keep the protoplasm in the connecting strand immobile is a measure of the motive force responsible for the protoplasmic streaming. Such a counter pressure has been termed "balance-pressure" by Kamiya.

The graphical representation of the motive force, and therefore the vital protoplasmic force, changes spontaneously with a definite period. The range of the change is usually ± 25 cm of water column. Instantaneous values of the balance-pressure, taken every 10 or 20 seconds, and plotted against time, yields

undulating curves which are called dynamoplasmograms (DPGs). They reveal characteristics as to frequency, amplitude and form of the wave in mechanical activities of the protoplasm.

Gelation of Protoplasm and its Bearing on the Periodic Functioning of the Motive Force Generation

When we admit carbon dioxide gas diluted to proper concentration to both blobs (*a* and *b*) of the plasmodium, the protoplasm in the double-chamber gels after a while abruptly. The protoplasm once gelated solates again when carbon dioxide gas is replaced with fresh air. Sometimes solation takes place even spontaneously in the carbon dioxide gas provided the gas concentration is not too high.

In Fig. 2 is shown a case in which the protoplasm was treated with 10% CO_2 , a rather low concentration for inducing gelation of protoplasm. The protoplasm frequently does not set to the state of gel at this level of concentration.

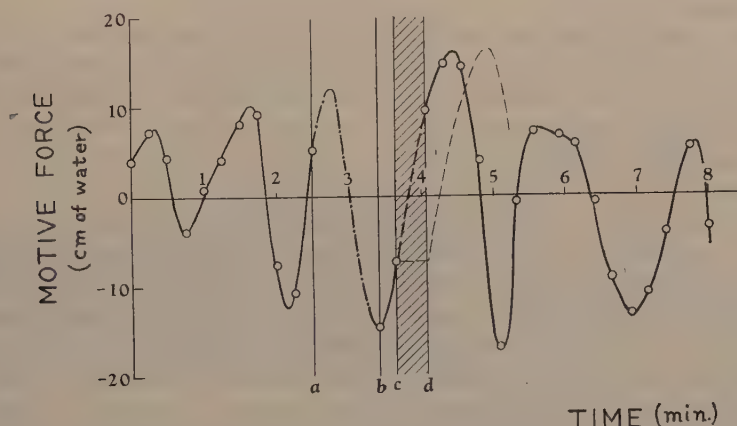


Fig. 2. Dynamoplasmogram showing the behaviour of the motive force generation before and after gelation of the protoplasm. The period in which the protoplasm sets to the state of gel is indicated by a shadow. During *a* and *b* the measurement was suspended in order to replace the air in the double-chamber with 10% CO_2 gas.

The first part of the undulating curve in Fig. 2 shows the normal rhythmic generation of the motive force. During *a*—*b* in Fig. 2 the measurement was interrupted in order to replace the air in the two compartments with 10% CO_2 gas¹⁾.

1) The period between *a* and *b*, during which time the curve was interrupted, was required for bringing three stop-cocks C_1 , C_2 , C_3 to the positions \vdash , \perp and \dashv respectively. After this procedure 10% CO_2 gas diluted with air was perfused through the two compartments, gas being let in at one end and let out at the other. After 30–60 seconds' perfusion, stop-cocks are brought back to the original position which are shown in Fig. 1. Oil drops d_1 and d_2 prevent CO_2 gas in the compartments from diffusing out and yet they are not in the way for establishing the pressure difference between the two compartments. This is because vent 1 is kept open to the atmospheric pressure and vent 2 is lead to the pressure controller, and the two drops work so to speak as frictionless pistons.

The protoplasm was free to flow during that time. After returning the stop-cocks to the measuring positions, the motive force was measured again at *b* and on. Though protoplasm was treated with 10% CO₂ in its entirety in this case, it behaved still quite normally for a while in regard to the streaming. But before long it changed its behaviour all of a sudden; it gelled. That gelation took place was indicated by the fact that no passive flow could be induced along the connecting strand even when pressure difference was established between the two compartments. When the endoplasm is in a state of sol, this is by no means the case.

Gel state of protoplasm, however, lasted only a brief period of time. The protoplasm solated again spontaneously as if the organism accommodated itself to the milieu containing CO₂. Immediately when the flow starts again, measurement of the motive force can be resumed. The curve (dynamoplasmogram) thus obtained is composed of a part covering the normal activity before carbon dioxide was applied and of another part covering the activity after recovery. Only during the period *c-d* which is shaded in the graph, the protoplasm remained in a state of gel.

In Fig. 2 special attention must be paid to the phase relation of the waves before and after gelation. It is clearly noticed here that the waves that come after *d* are found just in such a phase as would suggest *a continued periodic activity during the gelled period*. The curve marked with a broken line between *c-d* has been drawn simply by inferring from the curves before and after the interruption.

If the rhythmic functioning were stopped during the period in which the whole protoplasm was in a state of gel, the protoplasm would take up after recovery the phase which was left off just before gelation. Graphically, in this case, the waves after recovery should be somewhat like those shown in a thin broken line.

The fact we obtained above gives sufficient grounds for deriving the conclusion that the phase of the periodic activity generating the motive force was kept advancing while the protoplasmic flow was at a complete standstill. In other words, the periodic mechanism controlling the generation of the motive force must have been functioning no matter whether the protoplasm was free or arrested, no matter whether it was in a state of sol or gel.

Periodicity in Gelled Protoplasm as Revealed by Bioelectric Phenomena

A further proof of the above conclusion is to be had in figuring out the electric activity when gelation takes place all over the plasmodium.

Kamiya and Abe (1950) developed a method for studying simultaneously the potential difference (P.D.) between the two ends of the plasmodium and the motive force of the protoplasmic flow. They found that the two undulating curves representing the P.D. (electroplasmogram, EPG) and the motive force of the protoplasmic flow (dynamoplasmogram, DPG), have exactly the same period and are closely correlated in respect to wave form and amplitude, though the phase of the EPG invariably lags behind that of the DPG by about a quarter of the

period. This technique is applicable to the protoplasm treated with carbon dioxide.

When protoplasm is brought to the state of gel under the effect of carbon dioxide, the motive force of the protoplasmic flow can no longer be measured, but the P.D. is measurable independently of whether the protoplasm is sol or gel. Fig. 3 represents the result of the experiment in which gas mixture of

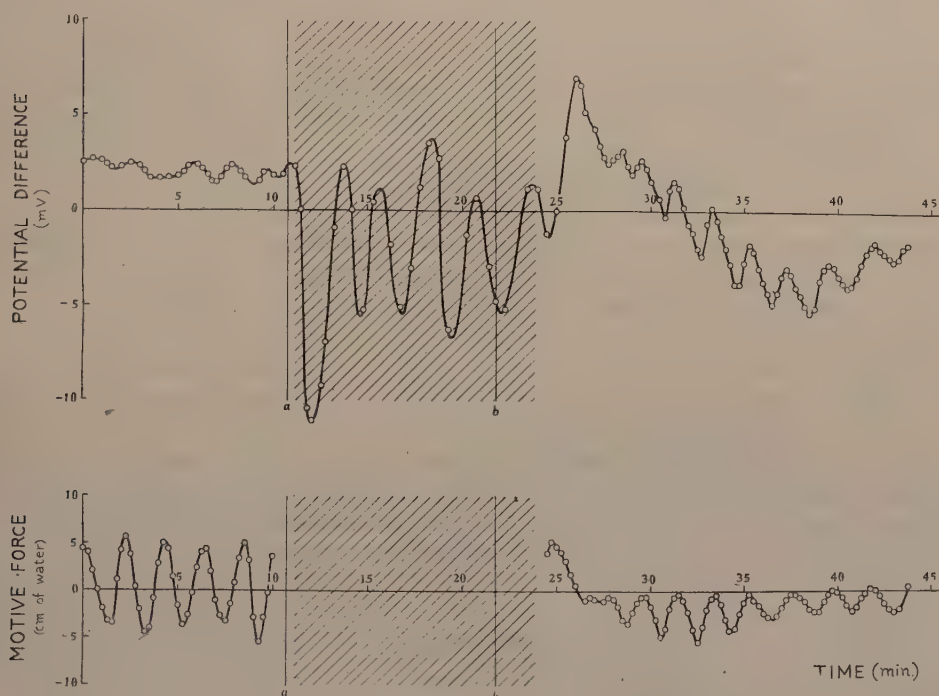


Fig. 3. Electroplastmogram (top) showing the periodic activity in the electromotive force generation while the protoplasm is in a state of gel, the duration of which is indicated by a shadow. Dynamoplastmogram (bottom) is interrupted during gelation.

90% CO_2 + 10% air was admitted to the protoplasm at the vertical line designated as *a*. Under the treatment with such a high concentration of CO_2 , setting of the protoplasm takes place without delay. No spontaneous recovery takes place, as was the case in the foregoing experiment, under as high a concentration as this. When, however, CO_2 gas was replaced again with fresh air at the second vertical line designated as *b*, the protoplasm began to solate before long without any observable ill effect. A few minutes after fresh air was substituted for carbon dioxide, the measurement of the motive force of the protoplasmic flow could be resumed. From the lower curves (DPGs) before and after gelation, it is impossible to infer any curve, since the interruption of dynamoplastmometry due to gelation is too long. But the upper curve (EPG) shows most clearly that rhythmicity, far from disappearing, was conspicuously amplified during the period in which the whole protoplasm was in a state of gel. Though it is not

within the scope of the present paper to discuss the reason for the augmentation of amplitude of the EPG under the influence of carbon dioxide, the curve manifestly confirms in terms of bioelectric activity, the conclusion derived from the foregoing experiment that the rhythmic activity is kept going even when protoplasm is kept motionless in a state of gel.

Concluding Remarks

The double-chamber method shows that the motive force of the protoplasmic streaming is generated alternately changing its direction, even though the streaming is arrested for hours. From this fact Kamiya (1942) was led to believe that the displacement of protoplasm due to the streaming can play no part in the causal chain of the mechanism of the reversal.

The present experiments not only confirm unmistakably the view held by Kamiya but also they further demonstrate that the rhythmic activity is operative even in the protoplasm which is entirely gelated. Thus in the light of the above findings, we may discard the view postulating possible physical or chemical changes resulting from the change in distribution of protoplasm as the cause of the reversal. Periodic functioning has its origin in a deeper source.

Kamiya, Nakajima and Abe (1954) found that the energy source required for the protoplasmic streaming in the slime mould is adenosine triphosphate which is formed mainly by fermentation. But there is no reason to assume that the periodic generation of the mechanical energy must have its counter-part in the fermentation process. The problem where the mechanism of oscillation is located in the interplasmic chain reaction will await further study.

Summary

The rhythmic functioning of protoplasm in the myxomycete plasmodium which is so strikingly demonstrated by the protoplasmic motion, has been confirmed to be operative no matter whether the protoplasm is in a state of sol or that of gel. Neither the flow of protoplasm nor the possible physical or chemical changes consequent to it play a direct part in causing the reversal of the streaming direction. Rhythm is a more deep-seated attribute of protoplasm.

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Sugar-containing Sap in the Flower-buds of *Cosmos* and *Coreopsis*

By

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While investigating the anthochlor pigments of *Coreopsis lanceolata* and *C. saxicola*¹⁾, the author's attention was drawn to the presence of watery sap which filled the interior of young buds covered tightly with, in general, eight inner involucre scales. This phenomenon was also found in the case of *Coreopsis Drummondii*, *C. tinctoria*, and *Cosmos bipinnatus*. It was, however, not found in *Cosmos sulphureus* and in other genera of the tribe *Heliantheae*, to which *Helianthus*, *Bidens*, *Dahlia*, etc. belong. Apparently this quite particular phenomenon has, however, some analogues in other plants, that is the so-called water-calyx (Wasserkelch) and water-capsule (Wasserkapsel).

In 1890 M. Treub²⁾ described for the first time the water-bud or water-calyx in the flower-bud of *Spathodea campanulata*, a tropical plant belonging to Bignoniaceae. Afterwards such water-calyces were described by G. de Lagerheim³⁾ in *Iochroma macrocalyx* (Solanaceae), by M. von Raciborski⁴⁾ in *Heterophragma adenophyllum* (Bignoniaceae), by G. Kraus⁵⁾ in *Parmentiera cerifera* (Bignoniaceae), and by S.H. Koorders⁶⁾ in 9 species of the families Solanaceae, Bignoniaceae, Verbenaceae, Scrophulariaceae, and Zingiberaceae. Such example was also found by H. Hallier⁷⁾ in *Leea amabilis* (Vitaceae). All of these plants are of tropic regions.

Of the plants indigenous or most commonly cultivated in Japan, K. Shibata⁸⁾ found the examples in *Campsis grandiflora*, *Catalpa Kaempferi*, *Clerodendron squamatum*, *C. trichotomum* and *Nicandra physalodes*. The water-calyx of *Stictocardia tiliaefolia* (Convolvulaceae) described by N. Svedelius⁹⁾ is somewhat different, because it develops during the postfloral period. H. Molisch¹⁰⁾ found that *Aconitum variegatum* (Ranunculaceae) growing wild in Europe, has water-calyx, while other species of the same genus, *A. napellus* and *A. lycoctonum* do not. Lately another example was discovered by F. Maekawa¹¹⁾ in *Codonopsis lanceolata* (Campanulaceae) (Table 1).

K. Shibata⁸⁾ found an analogous phenomenon in the capsule of *Firmiana platanifolia* (Sterculiaceae), and detected a considerable amount of gallic acid and a small amount of acetic and malic acid in the brown sap contained in it. It is very interesting to note that the sap contained in the unopened capitulum of *Cosmos* and *Coreopsis* showed a sign of existence of such organic acid even in a trace, but it strongly reduced the Fehling's solution, suggesting the existence in a considerable amount of reducing sugars in it. According to Shibata even a trace of sugar was not to be detected in the sap of *Firmiana*, and Molisch also described that in the sap of water-calyx of *Aconitum* no sugar could be found, though he found a yeast and spores of a hyphomycete subsisting and

germinating in the sap. The sap of *Clerodendron trichotomum*, though it was a laborious work to collect a few cc. of it, reducing substances could neither be found by Fehling's solution nor by paper chromatography. In sharp contrast with these examples the sap in the capitulum of *Cosmos* and *Coreopsis* contains sugars.

Table 1.

	Families	Species	References
Water-calyx	Campanulaceae	<i>Codonopsis lanceolata</i>	11
	Bignoniaceae	<i>Campsis grandiflora</i>	8
		<i>Catalpa Kaempferi</i>	8
		<i>Crescentia Cujete</i>	6
		<i>Heterophragma adenophyllum</i>	4
		<i>Kigelia pinnata</i>	6
		<i>Parmentiera cerifera</i>	5
		<i>Spathodea campanulata</i>	2
		<i>Stereospermum hypostictum</i>	6
	Scrophulariaceae	<i>Ilysanthes</i> sp.	6
	Solanaceae	<i>Ichroma macrocalyx</i>	3
		<i>Juanullosa parasitica</i>	6
		<i>Nicandra physalodes</i>	6
	Verbenaceae	<i>Clerodendron Minahassae</i>	6
		<i>C. splendens</i>	6
		<i>C. squamatum</i>	8
		<i>C. trichotomum</i>	8
	Convolvulaceae	<i>Stictocardia tiliifolia</i>	9
	Vitaceae	<i>Leea amabilis</i>	7
	Ranunculaceae	<i>Aconitum variegatum</i>	10
	Zingiberaceae	<i>Alpinia</i> sp.	6
Water-capsule	Sterculiaceae	<i>Firmiana plataniifolia</i>	8
	Compositae	<i>Coreopsis Drummondii</i>	
		<i>C. lanceolata</i>	
		<i>C. saxicola</i>	
		<i>C. tinctoria</i>	
		<i>Cosmos bipinnatus</i>	

The watery sap of *Cosmos* and *Coreopsis* buds was of pH 5.6–5.8 and its quantity in each bud is 0.05–0.15 cc. in the case of *Coreopsis lanceolata*, *C. saxicola*, and *Cosmos bipinnatus*, and 0.01–0.02 cc. in *Coreopsis tinctoria* and *C. Drummondii*.

When the sap was evaporated to dryness in a glass-dish on a boiling water-bath, a residue, making 1 to 1.5% of the sap, was obtained. It was then dissolved in 1/10–1/20 the original volume to give a solution convenient for the detection of sugars by means of paper chromatography. This solution thus pre-

pared was also used for qualitative as well as for semi-quantitative determination. For the purpose of determining the total reducing power of sugars, the Sumner's method¹²⁾ using 3,5-dinitrosalicylic acid as oxidizing agent was applied. For this purpose, 1 cc. of the sap was diluted up to 10 cc. in a measuring flask and with 1 cc. of this solution the sugar was quantitatively determined. It was found that 0.6–1.0 mg. fructose or glucose was contained per 1 cc. of this solution.

Though the quantity of sugars varied considerably by various factors, it was found that in *Cosmos bipinnatus* the concentration of fructose was 0.3–0.4% and that of glucose 0.1–0.2%, and in *Coreopsis* the concentration of fructose and glucose was 0.4–0.5% and 0.05–0.1%, respectively. Sucrose was not detected even in a trace either in *Cosmos* or *Coreopsis*.

Usually, an unknown reducing substance ($R_f=0.04$, *n*-butanol-acetic acid-water, 4:1:2, Whatman No. 1 filter paper, 20°) was found. This substance proved to be not identical with any of lactose, maltose and glucuronic acid, though the R_f value lies closely to those of these compounds. It was not an ordinary oligosaccharide and does not suffer hydrolysis by acid. This substance could not be, however, otherwise detected in any part of the capitulum. The parts of the capitulum and the stem of *C. lanceolata* contained sugars as shown in the following table.

Table 2. Distribution of sugars in the capitulum and the stem of *Coreopsis lanceolata*.

	Fructose	Glucose	Sucrose	An unknown substance
Ray flower	++	++	—	—
Tube flower	++	++	+	—
Involucrum	++	++	+	—
Stem	++	++	+	—
Sap	++	+	—	+

In certain plants, there are really found special hydathodal hairs as in the postfloral calyces of *Stictocardia* described by Svedelius and clavate hairs on the inner wall of the capsule of *Firmiana* by Shibata. No evidence has been obtained of the real excretion in the young buds of the present cases. Less specialized long hairs are, however, commonly found on the top region of the involucre scales and on the outer surface of flower petals of both ray and tube flowers, which are most probably serving for the sap excretion.

In short, it is not clear from what organs or tissues on the inner surface of the flowers of *Cosmos* and *Coreopsis* the sugary sap is exuded. So it is desirable to continue the investigation when further sufficient supply of materials becomes available.

The author desires to express his hearty gratitude to Prof. Shizuo Hattori for his kind advice during this work.

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A Theoretical Analysis of the Succession Process of Plant Community, based upon the Production of Matter

By

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The succession of plant community is always mentioned as one of the most interesting final problems in the studies of plant community. Since the publication of the comprehensive study of Clements 1916, we used to see schematic diagrams of plant succession in almost all descriptions on the local plant communities. The diagrams, however, show only the superficial succession series of plant species, or plant name, but nothing on the process of this phenomenon. For the scientific studies, the logical explanation of the process and the general law may be not less important than each observed result.

In the phyto-ecological study, we must always treat plants and their environment as opposite things, because the first object of the plant ecology is the causal explanation of the mutual relationships between plants and their environment, though naturally the plants are the prime entities. The plant community is able to develop itself on the basis of mutual actions and reactions between plants and their environment. The mutual actions and reactions are always working uninterruptedly, but we must cut off their working process in an instant from the continued whole process, in order to treat logically the developmental process of plant community. Actions of the environment and functions of plants are so intricate as we can not pick up each factor from the whole process without considerations on the mutual influences, but we dare to analyse the process into the plant functions and the environmental conditions opposed to them. These analysed factors, however, must be synthesized once more for the sake of formation of the logical images for the concrete phenomena in plant community.—The embodied synthesized process is no more than the growth (in the widest sense) of plants.

As an initial step to the study, we must pick up the principal plant function and principal environmental condition out of innumerable factors. The former for the growing plants is naturally the assimilation, especially the CO_2 -assimilation, or photosynthesis for the green plants (see Boysen Jensen 1932), and on the basis of this fundamental plant function the maintenance and development of the whole ecosystem become just possible. Therefore, the principal function in the ecology must be photosynthesis and the principal condition light, although the serious factors in each habitat and at each time can be water, heat, nutrient salts, etc., respectively. These factors would rather work in a long time as the secondary factors modifying the principal light-assimilation factor.

In order to make clear the mechanism working in much complicated pheno-

mena, the most important technique is abstraction. As above discussed, among numerous plant functions and various environmental conditions photosynthesis and light condition must be at first abstracted as the principal function and condition, and also the structure of plant and plant community must be abstracted in relation to this function and condition. With these abstractions, we can barely find out the general law hidden behind each accidental phenomenon.

The relationship between the succession of plant community and the organic production was illustrated by Lüdi already in 1923, although it was merely qualitative. The succession of lakes has recently been discussed by Lindeman (1942), Hogetsu and Ichimura (1953) etc., from this point of view. But concerning the land vegetation, we have scarcely any work in this direction. So in the present paper, we intend to explain theoretically the fundamental process of the subclimax of grassland, the destruction of grassland by trees shooting out of it, and the reproduction of young trees in forest, on the basis of dry matter production and of growth of plants computed with plant and plant community models, which are very simplified in regard to photosynthesis and light condition.

1. Subclimax of grassland formation

Vast grasslands develop here and there in the mountain region, especially on the lower slopes of volcanoes, in Japan. The general climate in Japan allows the thriving of the forest, for it has plentiful precipitation [annual precipitation; 800 mm (in northern part) —4000 mm (in southern part), in general 1500—2000 mm], and moderate temperature [mean annual temperature, 5°C (in northern part)—17°C (in southern part), in general 10—15°C]. The grassland, however, is not so easily succeeded by the forest, and continues its dominance for a long time. The continuation of grassland is almost always kept up artificially by mowing, grazing, and fire, etc. However, we can also regard as one of the most important causes of the autogenous maintenance of grassland the very thick growth of herbs, which always compete and sometimes overwhelm the tree seedling and yearling growing on grassland. The forest formation cannot directly succeed the flourishing grassland in optimum conditions, but it rather succeeds somewhat poor or degenerate grassland. On this point we must discuss more in detail in the following.

The succession from grassland to forest is achieved practically by the growing up of a number of tree seedlings on the grassland. The growth and development of seedlings is supported only by the positive economy of carbon assimilation. The light intensity in the herb community is very weak as reported in the previous paper (Monsi and Saeki 1953, p. 29–30). The relative light intensity observed in well developed herb communities was only 2–3%. Under these light conditions the carbohydrate economy of tree seedlings frequently becomes negative.

The rankness of plant community is able to be shown quantitatively with two characteristics, i.e. the height and density of the constituent plants. The density can indirectly be represented by the degree of light extinction in the plant community. Light is extinguished here mainly by absorption of leaves,

and the relation between light intensity and leaf amount is fundamentally represented by the following formula,

$$I = I_0 e^{-K F} \quad (\text{see Monsi and Saeki, 1953 p. 33}),$$

where I is the light intensity on the soil surface in plant community, I_0 the initial light intensity, K the extinction coefficient, F the leaf area layer (Watson's leaf area index), which means the ratio of the total area of leaf blades of a stand to the land surface covered with the stand. The extinction coefficient differs with the inclination, arrangement, and transmissibility of leaves; it is generally 0.3–2, and by horizontal leaves distributed at random theoretically and practically 1.

The first development from denuded land to grassland is mainly characterized by height growth and aggregation of migrated plants. A newly started plant community does not reach its temporary stability for several years, therefore, the seasonal change of vegetation must be considered. However, in order to discuss the principles of succession process in the simplest form, we assumed that a plant community of a constant density continuously grew in height without any seasonal change—plant communities of several kinds of density were here also considered—, because in the deciduous tree the assimilation and, therefore, the growth in winter are nought, and also in the evergreen they may be so small as we can neglect the values compared with those in summer (after Saeki's unpublished data). The increase of the density resulted by the aggregation of the constituents was represented with the growth curve of tree in the herb community of higher density.

It is very clear with the stratifying clip method (Monsi and Saeki 1953) that the leaf distribution of many plant communities generally has a common pattern. In the depth of plant community, there remain only a few leaves, for the most of leaves turn yellow and fall, or leaf development is poor in the darkness or as a result of deficiency of productivity. Leaves distribute maximally at the slightly inner part of the community. However, in the present paper we supposed that the leaves distributed homogeneously from top to bottom in the plant community, as the light intensity diminished exponentially with the depth of plant community.

The growth of trees is generally in the initial stage slower than that of grasses and forbs. Therefore, the photosynthesis of young trees in grassland formation must be hindered in the low illumination under the overtopping herbs. The dry matter productivity of young trees is mainly determined by the degree of coverage of herbs. The influence of herb community is separable into two main components, the one is the difference between the height of the herb community and that of the young trees, and the other is the density of the former. The density of herb community was represented here with the depth where the light intensity was only 10% of that in the open. The observed depth of 10% light was in general 20 cm–100 cm, or in the relative depth 2–10. In case of forbs with horizontal leaves it was small, and was large in grasses with erectly elongated leaves (cf. Monsi and Saeki 1953, p. 35 ff.).

As the value of plant productivity we used the daily means of CO_2 -assimila-

tion of sun and shade leaf calculated from Boysen Jensen's data in birch, ash, and beech (1932, p. 44-45), as in the previous paper (Monsi and Saeki 1953 p. 41). The productivity of young tree in herb community is determined as above

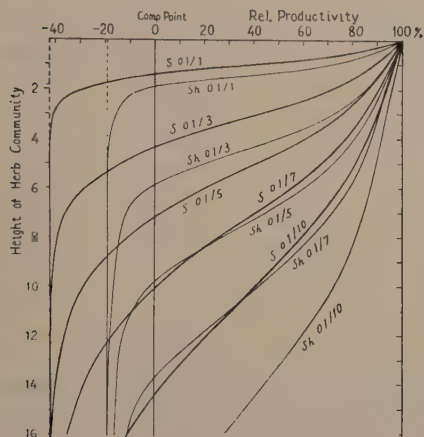


Fig. 1. The productivity curve of sun (*S*) and the shade (*Sh*) leaf in the foliage of herb community. The density of herb community is represented with 0.1/1, ... 0.1/10, those mean the relative light intensity of 10% occurs at relative height of 1, ...10.

mentioned by the light intensity which the young tree receives through the leaf canopy of herbs, and the light intensity is determined by the density of herbs and the height difference between two life forms. If the leaf density is homogeneous in a herb community, we can correlate the productivity of trees in a herb community with the height difference, as in Fig. 1 illustrated. The productivity decreases somewhat slowly within a small range of the height difference, while the height difference becomes large, the depression of productivity is more strikingly. The productivity of the shade leaf decreases naturally slower in weak illumination than that of the sun leaf. The compensation point of the sun and the shade leaf is reached at the depth where the relative light intensity is 3.72 % and 1.07%, respectively.

We assumed that all young trees of same height had same growth potential, but the real growth in height during a period was proportional to their relative productivity. This assumption is not so irresponsible, for it is well known that, when dominant trees of a forest fall down, young trees suppressed in the forest shadow can recover their growth and vigorously grow up just as trees having grown in full day light.

Further, for the more precise analysis of plant growth we must also regard the dry matter consumption of aphotosynthetic organs, such as stem, roots, etc., and the development of photosynthetic and aphotosynthetic organs for the production in the next period, too. The amount of plant growth is not merely proportional to the productivity of leaves, but it is decided by the balance between the production and consumption, and by the development of organs. The plant must be analysed as a system of dry matter production, or its growth as a development of dry matter reproduction. We treated here, however, the growth of tree was simply proportional to relative productivity, because we could find out more easily the fundamental law with this simple assumption, for the construction on the basis of the reproduction system was too complicate in order to calculate the amount of plant growth in this theme.

After these arguments and assumptions, we can discuss diagrammatically the fate of tree seedling and yearling in a herb community, as in Fig. 2 illustrated. The height growth curve of herb community is represented with *H*, that of young tree in the open with *L*, and in the herb community with *L'*, and the

height of herb community and young tree in it at the time t_n with H_{tn} and L_{tn}' . The new height growth ($\Delta L_{tn}'$) of young tree in the herb community in a short period Δt will be determined on the diagram by means of the assumption above mentioned. At first, the growth of tree of which height is as the same as L_{tn}' , under full light condition (ΔL_{tn}) is to be determined by the sliding of L_{tn}' upon the L -line and by the determination of the height increase on the L -line in Δt . Also the relative productivity is to be determined by means of Fig. 1 with the height difference between the herb community and the young tree ($=H_{tn} - L_{tn}'$). The growth of the young tree in the herb community ($\Delta L_{tn}'$) is $\Delta L_{tn} \times \text{rel. productivity}$, and it always changes the light condition for the dry matter production of the young tree itself in better situation. After the repeating of these calculations and drawings as in Fig. 2, a continuous growth curve of a young tree under the light condition being changed with the growth of the herb community and the young tree in it, can be constituted.

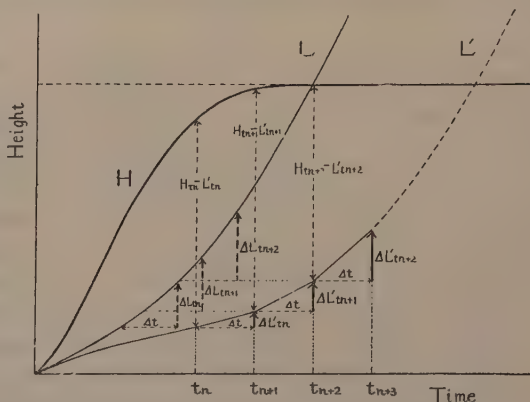


Fig. 2. Construction manner of the growth curve of a tree under a coverage of herb community. H is the growth curve of the dominating herb community, L that of a tree in the open, L' that in the herb community. See also the text.

In a herb community with high density, e.g. in which 10% relative light is already reached at the relative depth of 6 or smaller—we show this density briefly with 0.1/6—, the productivity of the young tree soon becomes negative and the plant perishes with the lapse of time, as the intensity of light falling on the young tree decreases rapidly in proportion to the increasing of height difference between the two life forms (Fig. 3). In the herb community of 0.1/6.5, the productivity of young tree is very small, but it remains continuously positive. So the young tree can maintain its life, and after the herb community reaches its ultimate thriving, the light condition becomes continuously, but very slowly, better for the dry matter production of the young tree as the young tree grows in height, though under natural conditions the young tree may soon or later be killed by various damage, e.g. fungus infection, etc.

In the herb community with density smaller than 0.1/7, the young tree can recover its growth very soon after a short suppressed time. From the herb community of 0.1/7 it already shoots out after ca. 9 periods (1 period corresponds about 1 year). In a herb community of 0.1/10, the tree growth is retarded in comparison with that in the open by only one period. In this analysis we see very clearly that the fate of a tree seedling in a herb community is very sharply controlled by the density of the dominating herb community. If the density is 0.1/6.5, or the light intensity at the relative depth of 10 is 2.9%, the seedling

has to die away, but if the density $0.1/7$, or the light intensity at the same depth 3.8% , the seedling is able to stand. These contrasts may more sharply appear under natural conditions, for on the one hand the dry matter consumption of aphotosynthetic system accentuates the deficiency of carbohydrate economy in weak light, and on the other it is highly possible that various damage attacks the weak plants more times than the healthy ones.

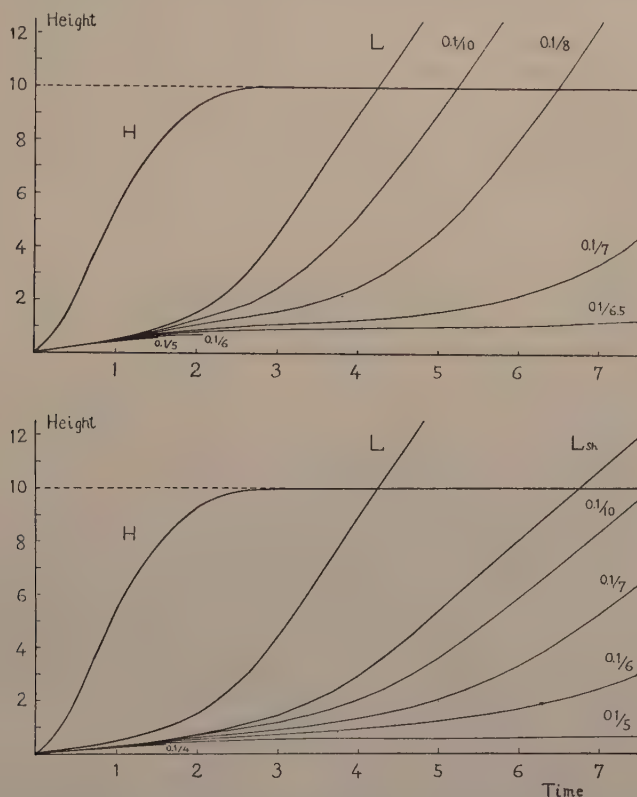


Fig. 3. Growth curve of the sun (upper) and shade (lower) leaf tree in herb communities of various densities. See the text and also Figs. 1 and 2.

Also concerning the tree with shade leaves (see Fig. 3b), we can illustrate the similar growth curve as on the sun leaf tree in Fig. 3a, naturally the critical density of the herb community is in this case higher than in the case of the sun leaf tree. The density of herb community in which the seedling of shade leaf tree scarcely continues its life, is $0.1/5$, and this density corresponds to that of $0.1/6.5$ in case of the sun leaf tree. Under the full light condition, as the relative productivity of shade leaves is smaller than that of sun leaves, i.e. $56.3:100$, the growth curve of the shade leaf tree in the open induced from that of sun leaf tree with the correction of relative productivity, rises more slowly against the time abscissa than that of sun leaf tree, as it is practically well known in many kinds of shade trees.

The relative productivity of the tree in the herb community is determined by the height difference of tree and herbs, therefore, if the tree has relatively larger growth velocity, the recovering of its productivity under herbs is faster and it can tolerate the shadow in the closed herb community. In Fig. 4, we illustrate three trees with different growth velocities; the middle L_n is the same of L as in Fig. 3, and L_f and L_s have $1/3$ period earlier or later growth velocity comparing with L_n . Under the herb community of which density is $0.1/6.5$, L_f shoots out after 8 periods, but L_s already perished in about 3 periods.

Since the time when the herb community attains its ultimate height, the change of light condition caused by tree growth is not so dynamic, as in the growing period of the herb community. The critical density of herb community for survival of young tree is constant, i.e. ca. $0.1/7$ for sun leaf tree, and ca. $0.1/5$ for shade leaf one.

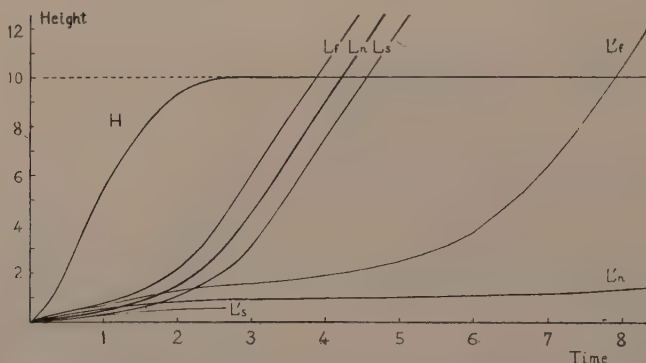


Fig. 4. Growth curves of sun leaf trees with three kinds of growth velocity.

Under full light condition, L_n is the same tree in Fig. 3a, L_f grows $1/3$ period faster than L_n , and L_s $1/3$ period slower. L_f' , L_n' , and L_s' are the growth curves in the herb community.

It is also recognizable that there is not only the intraspecific, but also inter-specific difference of tolerance in plants, though the real causes of tolerance are yet unknown. Therefore, the same condition is able to act to some species or individuals enough bright to continue their life and growth, but to some others too dark to live in, so the tolerant species survive and make their community. However, it is conceivable that the fundamental relationship between young tree and herb community above discussed may be responsible for each individual of each species, with corrections regarding to specific productivity and growth rate.

One of the most important problems for the subclimax of grassland is the deviation of the density in one herb community, for, even if the mean density is very high, trees can shoot out on the points of low density, and at last they will destroy the grassland formation. On this problem we may discuss the detail in the following chapter.

2. Destruction of herb community by trees growing in it

When the tree has shooted out from the herb community, the above mentioned relationship between both the life forms becomes reverse. The herbs which have been dominating for a long time are now suppressed in the tree shadow, and they retire, or replacing them, more tolerant herb species make together the undergrowth of the new forest.

As the most simplified model of a tree crown we imagine a disk. Cone or sphere shaped model seems to be more suitable for this purpose, but we used here the simplest disk model, in order to sum up very easily the influence of the tree crown model on the productivity of undergrowth, without mutual interference of models themselves, and also because the shade of a disk can represent the fundamental characters of tree shade. The stem is temporarily neglected in the computation, for the shade of stem is almost covered by that of crown.

The light condition under a disk is changed in two manners, the one is the decreasing of diffused light, the other the shortening of sunshine duration. The pattern of light condition under a disk is the same, independent of the size of the disk, e.g. if the distance from the disk is measured relatively with the disk radius, the light intensity decreased by the disk is always equal at the same relative distance. For the purpose of simplicity, we assumed that the sun always passed through zenith from due east to due west, so the sunshine duration in the open was just 12 hours, and that the whole sky was homogeneously bright. The sunshine duration at a point under the disk was 12 hours minus the time shaded by the disk, or $12 \text{ h} \times \frac{\text{angle of disk to the point}}{180^\circ}$. During the time in

which the disk intercepts the direct sunshine, the point receives only the diffused light from the sky. It was assumed that the light intensity was on daily average 10 klux from blue sky, and as direct sunshine (without sky light) 40 klux, therefore, the light intensity in the open under direct sunshine was on daily average 50 klux. Under the cloudy sky, the average light intensity was assumed as 10 klux of diffused light. In the districts of higher latitude where the sun shines almost from lateral even in summer, these assumptions are, we think, too unpractical, and as the model of tree crown, the disk model must be changed for cylinder or cone, although the calculation should become very complicated.

The diffused light intensity at a point under a disk is able to be relatively calculated by means of the ratio of interception of sky light caused by the disk—mathematically the ratio of solid angle of the disk, of which the vertex is the point under the disk, to the whole hemisphere. The sphere of influence of a disk upon the diffused light is somewhat smaller than upon the sunshine duration (Fig. 5), and it is isotropic for diffused light, but anisotropic for sunshine duration, for the portion influenced by a disk upon the sunshine duration is limited only on a belt stretching from west to east with the same width as the disk diameter. On a horizontal plane under a disk, the influence upon sunshine duration and diffused light is strongest at the point on the axis downward from the disk centre, and the influence must surely be maximal just under

the disk, then it becomes continuously weaker with increasing of the distance. However, out of the area directly under the horizontal projection of the disk, a minimum light intensity occurs at a point of a depth below the disk horizon.

The daily net production under a disk was calculated as follows:

on a fine day,

$$P_{nf} = [a_a \cdot t_a + a_{s+a}(12 - t_a)] - 24r,$$

on a cloudy day,

$$P_{nc} = a_a \cdot 12 - 24r,$$

where a_a and a_{s+a} were the hourly real photosynthesis (excluding the respiration) of unit leaf area under diffused light and total daylight, t_a photosynthetic time under diffused light, r the respiration. As the amount of hourly photosynthesis, the average values on sun and shade leaves of three typical deciduous trees, beech, ash, and birch, were used here also after the data of Boysen Jensen 1932, as before. Following are the cardinal values:

	Respiration $C_6H_{10}O_6$ g/m ² /h	Comp. point lux	Net Assimilation $C_6H_{10}O_6$ g/m ² /h	
			10 klux	50 klux
Sun leaf	-0.082	680	0.454	0.514
Shade leaf	-0.021	250	0.221	0.232.

The shading of a disk is so severe on the daily net production just under the disk, that it falls to negative value, but outward of some distance from the disk, only weak influence is expected (cf. Fig. 6), because only slight decrease of assimilation is brought with somewhat heavy depression of light intensity in higher illumination, although in lower illumination the assimilation varies conspicuously as the light intensity. The negative production region, therefore, is very limited, i.e. only 0.2 relative height even under the centre of disk, or the darkest position on a horizontal plane under the disk. When the height of disk is 1, the productivity already attains under the centre of the disk ca. 80% of that in the open. By means of these calculations, it will be easy to understand that the severe influence on undergrowth of an isolated tree is recognizable only just under the tree crown, or only the herb community of almost the same area as occupied by the tree crown may be destroyed when a tree is shooting up from the herb community. As the tree crown, however, becomes enough high, the herb community can recover from the suffering.

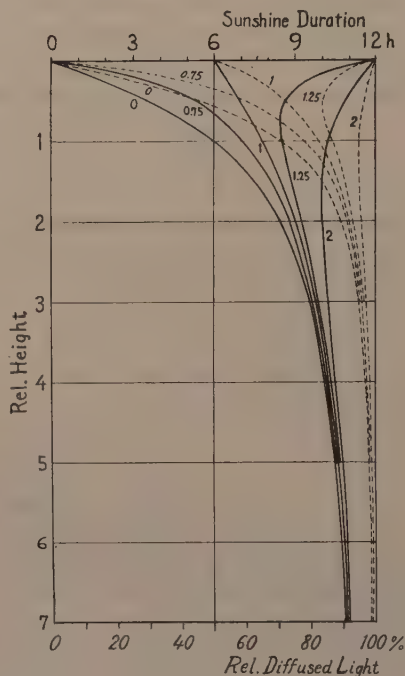


Fig. 5. Light condition in the shadow of a disk. Relative height is measured by its radius (H/R). Thick solid lines represent the time of sunshine duration, broken lines the relative intensity of sky light. The figures by the curves mean the distance from the centre of the horizontal projection of the disk: the distance is represented as a relative value by the disk radius.

In this state of succession, the vegetation has physiognomy like a savannah, or it is familiar as park landscape. In order that the forest succeeds the herb community, few scattered trees which have grown up must be followed by a number of trees shooting out of the herb community and by aggregation of

trees. This will be made clear by calculation with a model community of numeral disks. The highest density of disks aggregated on a horizontal plane is reached by the ordination of disks which contact with each other without any distance of margin, leaving a small space among three disks, and consequently, their centres are regularly on regular triangles. The light intensity on the soil surface under the aggregated disks is certainly the highest at the centre of each small space, so we will mainly discuss the light intensity and dry matter production on this position. The direction of the disk ordination has a meaning for the reduction of sunshine duration,

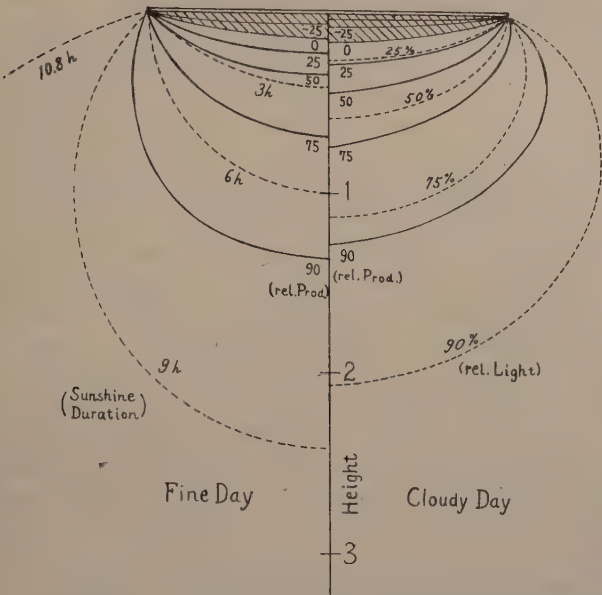


Fig. 6. The light condition (broken lines) and the relative productivity (solid lines) under a disk. The left half of the figure represents the values on a fine day, and the right half those on a cloudy day. Ordinate is the relative height of disk.

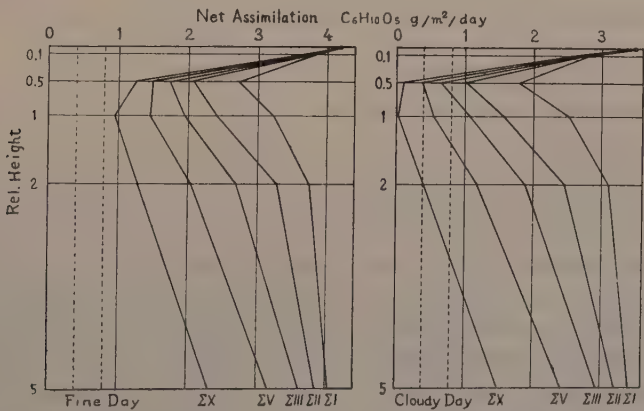


Fig. 7. The decreasing manner of net assimilation at the centre of the space of disks. East-west ordination. ΣI, ΣII, ΣX mean the number of rings of disks surrounding the space. Ordinate is the relative height of disks.

though it is not concerned in the decrease of diffused light. For example, in case of the ordination from east to west, the sunshine duration is shorter than in case of the ordination from north to south. The manner of depression of light intensity under the aggregated disks also changes with the height of disks.

The depression of light intensity caused by aggregated disks brings naturally the reduction of dry matter production of the plant growing under the space of disks, and the latter becomes sometimes very serious with the increase of total number of the disks, though the range of the reduction of the dry matter production varies with the height of disks. For example, if the total number of disks is 300, or the number of rings of disks around the central space 10, and their height 1, the net production of the plant growing under the space amounts only 0.95 g/m²/day on a fine day (in the open 4.20 g/m²/day), and only 0.03 g/m²/day on a cloudy day (in the open 3.49 g/m²/day). If the amount of respiration of aphotosynthetic organs is assumed as the consumption of dry matter of two times or more of that of leaves, the plant growing under the space of the aggregated disks has to be perished, because the carbohydrate economy must be negative. The influence of the aggregation of disks is certainly under the cloudy sky severer than under the fine sky, for the initial light intensity is in the former weaker than in the latter. If the disk height is small (e.g. $H=0.1$), the productivity decrease is not so severe as the herb community is quite destructed, and out of some extent the more increase of aggregated disks is not concerned with the reduction of productivity of plant growing under the centre of space, for, when the height of disk is small, the influence of disk is very limited in the small range around the disk. In case of the disks at large height (e.g. $H=5$), the influence is certainly weak, but the depression of productivity continues with the increase of the number of the aggregated disks.

The process of the destruction of the herb community caused by the aggregation of trees will be made clear by means of the change of distance of disks of a definite radius (Fig. 8), or the radius at change of disks at a definite distance (Tab. 1). In regard to this point, we computed the productivity under the central space of 5 rings of disks, of which height was 1 and ordination in north-south direction. The influence of disks is very weak in case of the aggregation at a distance 2, i.e., the plant under the central space among disks shows

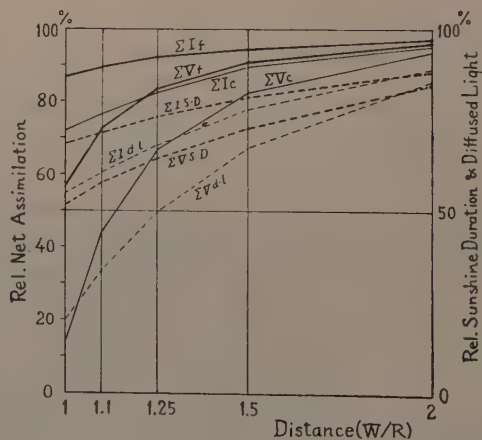


Fig. 8. Relationship between the distance of disks and the relative productivity of the plant growing under the central space. North-south ordination. The distance between disk centres is measured by the diameter of disk. The thick solid line represents the productivity on a fine day, the thin on a cloudy day. ΣI and ΣV mean the number of rings. Broken lines represent the light condition; S-D sunshine duration, d-l diffused light.

on fine day 96.3% productivity of the value in the open, and on cloudy day 93.4%. Or, if the radius of disks is 0.25, the productivity reaches 97.9% of that in the open on fine day, and 98.3% on cloudy day. The productivity decreases severely only by small distance, or large radius, and when a large number of disks aggregate. These computations will well suggest that, for the destruction of herb community by the standing forest, the closing of leaf canopy of trees, the number and density of trees growing up through the herb community have to be considered, and the sun herbs will abruptly disappear, when the trees reach near their maximal density.

Table 1. Productivity of the sun leaf tree at the soil surface under the central space of five rings of disks arranged in regular triangles (north-south direction). The diameter of disks changes from 1 (equal to the distance W between disk centres) to 0. The height of disks is equal to a half of the distance.

Dia- meter of disks ($2R/W$)	Sun or Dif- fused light (\checkmark)	Light inten- sity klux	Fine day				Cloudy day	
			Assm. $C_6H_{10}O_5$ g/m ² /h	Assm. time h	Gross Assim. $C_6H_{10}O_5$ g/m ² /day	Net Assim. $C_6H_{10}O_5$ g/m ² /day (rel.)	Gross Assim. $C_6H_{10}O_5$ g/m ² /day	Net Assim. $C_6H_{10}O_5$ g/m ² /day (rel.)
1	Sun Dif.	42.0 2.0	0.509 0.209	6.20 5.80	3.15 1.21	4.36	2.40 (57.1%)	2.51
0.8	Sun Dif.	44.9 4.9	0.510 0.355	7.47 4.53	3.81 1.61	5.42	3.46 (82.3%)	4.26
0.667	Sun Dif.	46.6 6.6	0.511 0.400	8.33 3.67	4.26 1.47	5.73	3.76 (89.6%)	4.80
0.5	Sun Dif.	48.1 8.1	0.512 0.429	9.35 2.65	4.79 1.14	5.93	3.96 (94.3%)	5.15
0.25	Sun Dif.	49.5 9.5	0.513 0.450	10.71 1.29	5.50 0.58	6.08	4.11 (97.9%)	5.39
0	Sun Dif.	50.0 (10.0)	0.514 (0.454)	12.00 0	6.16 0	6.16	4.20 (100%)	5.45
								3.49 (100%)

The number of the trees shooting out the herb community is decided by various factors, e.g. number of seeds fallen upon the ground, germination percentage, damage of fungi and animals, frequency of bright portion in which the light condition is better than the light minimum of tree seedling, etc. The frequency of bright portion was measured at some herb communities on the montane grassland in Kirigamine (1600 m above sea-level), Nagano Prefecture, middle Japan. Apparently homogeneous parts of *Miscanthus sinensis* community and *Arundinaria hirta*-*Sasa nipponica* community were selected for the station. The surveyed area was in the former 75 m², and in the latter 30 m². The area was divided into quadrats of 1 m², and in a quadrat the relative light intensity on the ground was measured by means of a photoelectric cell on a point appointed by the random numbers, parallel to this the light intensity in the open was decided by another photoelectric cell. The average standing crop of the

aerial parts of the herb communities was in the former 836 g (fresh weight)/m² of the photosynthetic system, or ca. 4.5 leaf area layers, and 616 g/m² of the aphotsynthetic. In the latter vegetation the plant community was not so close as in the former, and its standing crop was only 476 g/m² (ca. 1.9 leaf area layers) +228 g/m². After these procedures, we could get a frequency curve of light distribution, as in Fig. 9 seen. If the light minimum of tree seedlings may be 5%, the tree seedlings can be expected to stand up in the former community ca. 1/4 of the germinated seeds, and in the latter all of them. In reality, seeds and seedlings may be injured by fungus infection, wild small animals, etc., so the number of young trees which are growing up in the herb community becomes very rare. This must be complemented by a large number of seeds fallen on the ground. For these reasons, the pioneer plants which invade grassland are mostly sun plants with enormous production of seeds and rapid growth, e.g. *Pinus*, *Betula*, *Larix*, *Quercus*, etc.

As the results of the calculations and discussions above written, it is made very clear that the herb community which has formerly been dominating will be heavily destroyed by the succeeding tree community, just as a number of growing trees aggregate densely and their crowns make a leaf canopy closed enough; on the contrary, isolated trees have scarcely the destructive influence on the herb community. The deviation of light condition in the herb community and the number of seeds fallen upon, etc., are also important problems for the succession of the forest after the grassland.

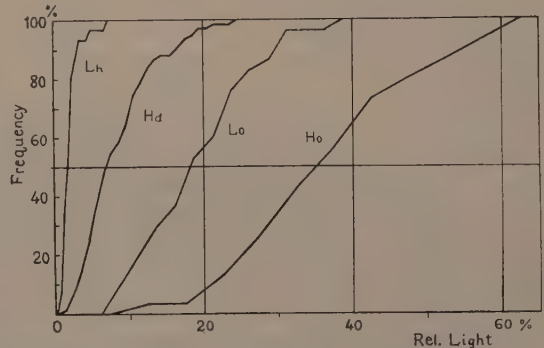


Fig. 9. Frequency curve of light intensity on grassland and in forest. Grassland, H_d : *Miscanthus sinensis* community, H_o *Arundinaria hirta*-*Sasa nipponica* community, in Kirigamine (1600 m above the sea), Nagano Pref. Forest, *Fagus crenata*-*Quercus mongolica* var. *grosserrata* community in Oze, Hukusima Pref. L_o above the undergrowth of *Sasa oseana*, L_h , under the *Sasa*-synsium.

3. Regeneration of young tree under forest canopy

The regeneration of the offsprings of dominating trees is very problem of the climax community. In the shade of vigorous forest, especially of shade trees, as well known, it is so dark that no new offspring can grow up in the darkness, for the light intensity under the forest canopy is even above the undergrowth 5–25%, and on the soil surface under the undergrowth only 1–3% (see Fig. 7 and also Monsi and Saeki 1953 p. 28).

In a matured forest, the tree crowns are held by high trunks and aggregate one another, so we assumed the forest canopy as a vast coverage on which numerous small gaps were scattered. The light intensity becomes below a

certain height in the forest, if the canopy is homogeneously closed, to have almost a definite one as well in horizontal direction as in vertical one, therefore, the light condition for young trees growing in the darkness of forest is scarcely changed with their growth. Their fate under this light condition will be decided, if we take up here only their life concerning the light condition, simply by their light minimum which may be determined by the annual balance between their production and consumption of matter.

Practically, special references must be made in the case of fall of trees belonging to the dominant class. After the fall of trees, a wide opening remains in the forest canopy, so the light condition under new opening becomes better for the dry matter production, consequently for the growth of young trees. If we assume the opening is strictly round shaped—a rugged opening may be replaced by a round one with some considerations—, the light condition under an opening has a pattern just reverse of that under a disk already discussed. We considered, however, in this case only vertical components of diffused light through the opening, because the light is accepted by plant leaves mainly horizontally arranged, while in the foregoing case light components in all directions were accepted by a plant under a disk. The intensity of the diffused light can be calculated after the following formula (Hirayama 1948, p. 379), i.e.

$$U = \frac{1}{2} \left[1 - \frac{(H/R)^2 + (D/R)^2 - 1}{\sqrt{\{(H/R)^2 + (D/R)^2 + 1\}^2 - 4(D/R)^2}} \right],$$

where U is the relative light intensity on a point under the opening, R the radius of the opening, H the height of canopy, D the distance of the point from the horizontal projection of the centre of the opening. The largest growth in the weak illumination under the opening naturally must be achieved at the brightest position, or under the centre of the opening, so we discuss the fate of young trees at this position. Therefore, the distance D becomes nought, the formula above cited becomes as simple as follows:

$$U = \frac{1}{2} \left[1 - \frac{(H/R)^2 - 1}{(H/R)^2 + 1} \right].$$

These formulae give us light decreasing curves which are nearly mirror images of the curves under a disk in Fig. 5.

The amount of the dry matter production under direct sunshine can roughly be measured by the photosynthetic time, so we calculate the sunshine duration as before. The average intensity of direct sunshine and of diffused sky light, and that on cloudy day, were respectively assumed as the same values as in case of the computation under the disk in the foregoing chapter. Also the computation of dry matter production was pursued according to the same formulae in p. 68.

For the discussion of the fate of young trees, we needed here the annual productivity, and used the arithmetic average of the total productivity on a fine and a cloudy day, for the ratio of fine to cloudy time is, though very roughly, one to one in middle Japan.

When the relative height (H/R) is smaller than 1.6 (rel. light intensity ca. 30%), the sun leaf can produce more dry matter than the shade leaf, but the

relative height becomes larger, the relation between the sun and shade leaf becomes reverse. The compensation depth is in the sun leaf 2.95 (ca. 10% light), and in the shade leaf 5.3 (ca. 3% light). The relative light intensity at the compensation point is somewhat higher than that in case of the discussion of subclimax of grassland, because the average absolute value of initial light intensity in this case is assumed lower than in case of subclimax of grassland, as we have not yet enough data to make the standard assimilation curve in the higher illumination beyond the range of 50 klux.

Numerous small gaps scattered on the forest canopy make the light condition somewhat better than that of above mentioned, where we assumed the canopy was completely continuous without any gaps. The relative light intensity on the forest floor is nearly the same as the percentage of the total area of gaps. The light intensity obtained in the forest generally ranges from 1% to 20%, so we discuss here the regeneration of young trees under the forest canopy with gaps of 1%, 2%, 5%, 10%, and 20% relative area.

If assumed the average light intensity in the forest to be $d\%$, the light condition at the centre under a new opening is as follows. The sunshine duration is

$$t_g = t_0 + \frac{12d(1-\alpha/180^\circ)}{100},$$

where t_0 is the time in which the centre receives the direct sunshine through the opening, α the vertical angle of the opening, of which vertex is the centre. We assume here the direct sunshine fallen on the soil surface through the gaps of the forest canopy has the same intensity as that of incident light, while the diffused light is weakened corresponding to the interception of the forest canopy. In reality, however, also the direct sunlight diminishes its intensity with dispersion of sun flecks, and consequently the sunshine duration on the soil surface is rather prolonged. A number of small gaps must be included in the new opening when trees fall down, so the component of diffused light through the remnant gaps diminishes to

$$d' = d \left(1 - \frac{\frac{1}{2}\pi - \text{solid angle of the opening}}{\frac{1}{2}\pi} \right) = d(1-c).$$

Therefore, the light intensity on the soil surface under the forest canopy with $d\%$ gaps becomes at the centre under an opening as follows,

$$\begin{aligned} \text{diffused light:} & \quad 10 \text{ klux } [U + d(1-c)], \\ \text{total sunlight:} & \quad 40 \text{ klux } + 10 \text{ klux } [U + d(1-c)]. \end{aligned}$$

By means of these light intensities, we can easily calculate the relative productivity of young tree growing under an opening of forest canopy with numerous small gaps, in the same manner as before. Naturally, the larger the total area of gaps on forest canopy is, the larger the productivity of undergrowth or young tree becomes. However, even if the light intensity in the forest is 20%, when the relative height of the opening (H/R) is over 3, the productivity of the shade tree surpasses that of the sun tree. In the forest, therefore, unless the opening is very large enough, the succeeding trees are highly probably belonging to the shade ones.

Using the productivity above discussed and a normal growth curve of tree, we can graphically analyse the growth of tree in the shade of forest after the similar method and with the same supposition as in the case of the discussion of growth in herb community. The productivity of young tree is decided by the light intensity falling upon the young tree, and the light intensity itself is determined by the dimensions of the opening and the height difference between the opening and the young tree. Under the forest canopy, the young tree grows in

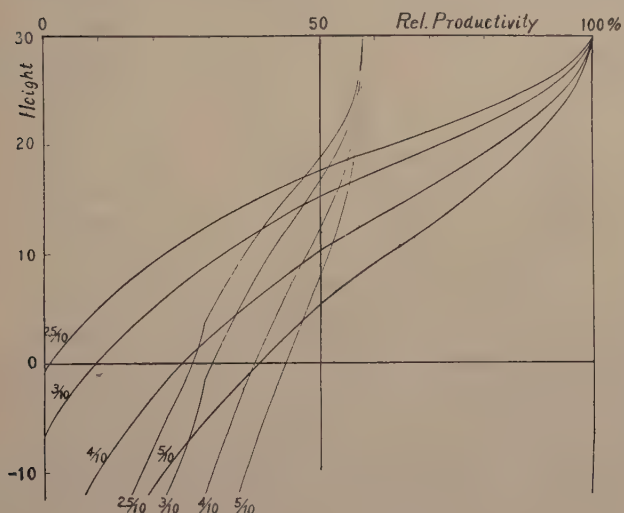


Fig. 10. The relative productivity under the centre of an opening of forest canopy with 5% gaps. The radius (R) of the opening is relatively shown by the height of the leaf canopy (H), i.e. 1.5/10, 3/10, 4/10, 5/10. Thick lines are of the sun leaf, thin lines of the shade leaf.

height proportionally to its relative productivity, and, when the young tree is brought in the open, it begins to have the same growth rate as that of the tree which has grown up in the open from the first. As the normal growth curve of sun tree, we used here that of a *Fraxinus* plenter-wood in Akita, northern Honsyu, Japan. The habitat condition of the regeneration is assumed that for the sun leaf tree the light intensity before the fall of dominant trees is 5%, and the relative radius of the opening (radius/height= R/H) 2.5/10, 3/10, 4/10, and for the shade leaf tree the light intensity 5% and 2%, and R/H 1.5/10 and 2/10.

The time in which the tree attains its ultimate height (=30 units, generally corresponds 30 m) is very different according to the conditions above assumed (see Tab. 2). In spite of the lower productivity of the shade leaf tree under full daylight,—the normal growth curve of the shade leaf tree introduced from that of the sun leaf tree rises naturally under full light condition slower than that of the latter—the growth of shade tree under forest canopy is rather larger than that of sun leaf tree, and it retards only slightly from its normal growth under full daylight.

In a forest just growing vigorously, the relation of the growth of young tree to an opening of the forest canopy is more complicated, as the height of the forest canopy becomes higher, and the dimensions of the opening smaller with the lapse of time. In order to make a clear analysis of this phenomenon, these two changes will separately be discussed in the following. When the dominating trees grow up, the opening also becomes higher, and even if the

Table 2. The time until the sun and the shade leaf tree attain their ultimate height under the light condition through an opening of forest canopy with small gaps. R is the radius of the opening, and H the height of the forest canopy.

Relative radius of opening R/H	Total area of small gaps	Sun leaf tree		Shade leaf tree	
		Compensation depth (m)	Years	Compensation depth (m)	Years
1.5/10	2 %			36	654
2/10	2 %			48	519
2.5/10	2 %			60	
"	5 %	30.6	700 or more		496
3/10	2 %			72	
"	5 %	36.8	433		475
4/10	5 %	49.0	335		451
$\infty/10$			250		424

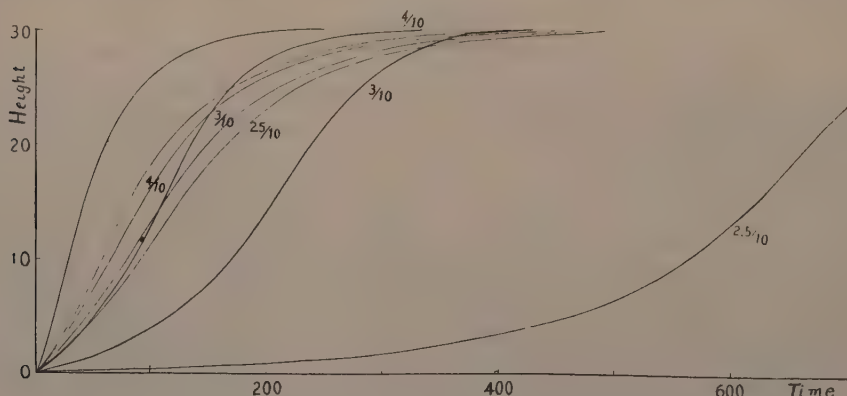


Fig. 11. Growth curves of the sun (thick line) and the shade leaf tree (thin line) on the soil surface under the centre of an opening of the forest canopy with 5% gaps. The opening is 4/10, 3/10 and 2.5/10. The curves without figures are the normal growth curves in the open.

dimensions of the opening remains constant, it becomes relatively smaller to the position of the young tree on the forest floor, so that the young tree which has begun to grow under the light condition better than its compensation point after the fall of some dominating trees, receives gradually less light, and its growth is sooner or later stopped, and at last the young tree must be withered. When the growth velocity of dominant trees is large, the depression of the light intensity is large and rapid. On the other hand, the young tree can improve the light condition by means of its own growth because of the diminishing of the height difference between two strata. The range of improvement, however, is very limited, and the light condition often becomes serious for the young tree, as the growth of the vigorous dominant trees is too large in comparison with

the suppressed growth of the young tree in the forest shadow, and the height difference between two generations becomes relatively so large, that the intensity of the light through the opening is diminished to the value under the compensation point of the young tree. The regeneration of young tree may be succeeded only in matured or over-matured forest, where the opening continuously hold its definite dimensions absolutely and relatively.

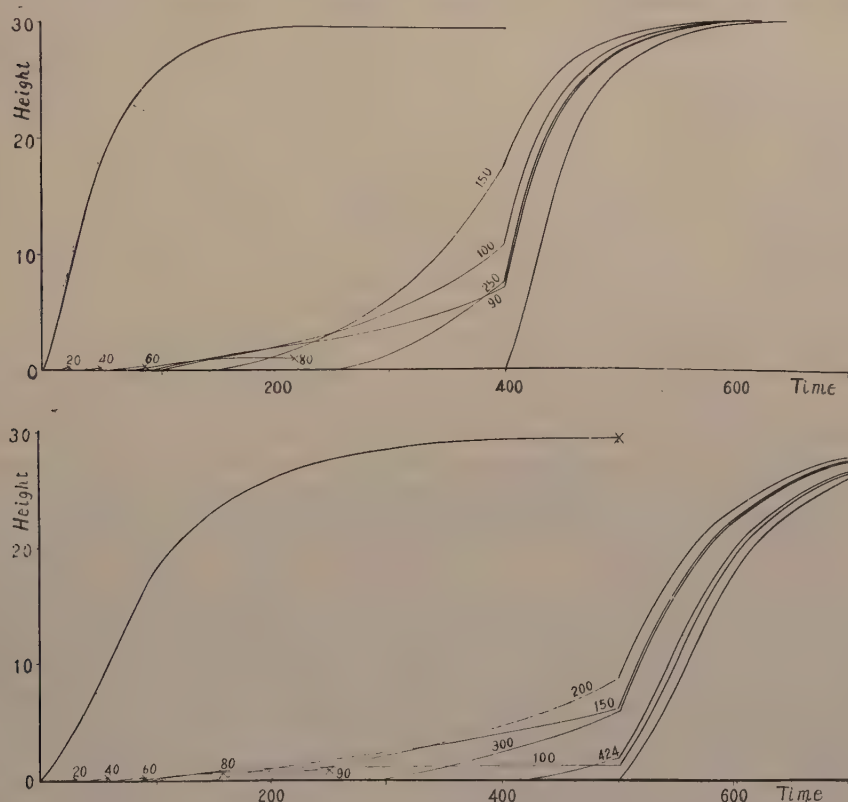


Fig. 12. The fate of young trees which begin to grow when the opening is made by the fall of trees in growing forest canopy. Upper: sun leaf tree under the opening of $R/H=3/10$, and the forest canopy with 5% gaps. Lower: shade leaf tree under the opening of $R/H=2/10$, and the forest canopy with 2% gaps. The figures by the curves show the time of start of growth, or of germination.

The stretching of tree branches in the forest canopy and the following reduction of the opening may have more serious influence upon the fate of young tree which has started to grow under the opening. The reduction of the radius of only 0.12 length unit per 10 periods kills the young tree in case of the opening of $R/H=3/10$. As the result of this calculation, it will be more probable that, when an opening is made by fall of trees in the canopy of a young forest, the opening will not be filled up by the suppressed young tree now recovering

its rapid growth, but rather by the lateral stretch of branches and twigs of dominant trees. A very large opening may be expected for the regeneration of young tree in unmaturing forest, because the light condition on the forest floor under the opening very rapidly becomes worse for dry matter production of young tree, as the dimensions of the opening is reduced absolutely by the extension and relatively by the rapid height growth of the dominant trees composing the forest canopy.

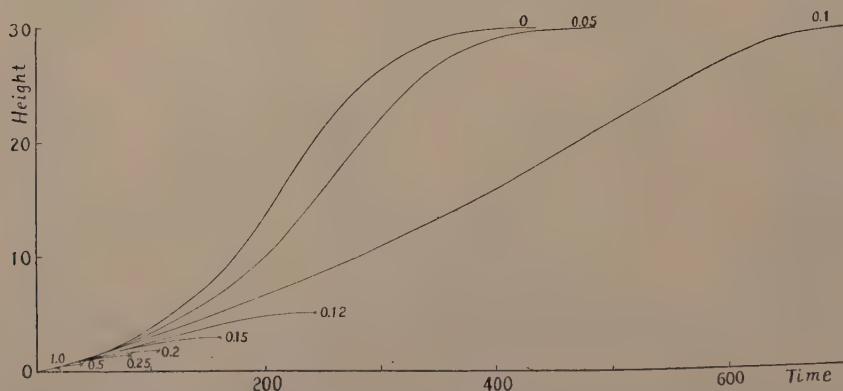


Fig. 13. The fate of young sun leaf trees, when the opening ($R/H=3/10$) of forest canopy with 5% gaps is closed by the stretching of branches around it. The figures by the curves mean the velocity of stretching of branches, e.g. 0.5 means the stretching is 0.5 unit length (ca. 0.5 m) in 10 time units (ca. 10 years).

In the forest, too, the deviation of the light condition is able to have a large meaning for the regeneration of young tree as in the herb community in sub-climax. So we measured the light intensity in a *Fagus crenata-Quercus mongolica* var. *grosseserrata* community in Oze, northern Honsyu, Japan (see Fig. 9), after the same method as in case of the herb community before mentioned. Above the undergrowth of *Sasa oseana*, it varied in a range of 6%–38%, and on the average 18%, but under the *Sasa-synusium*, it was only 0.2%–7.3%, and on the average ca. 2%, therefore, the undergrowth, especially the *Sasa-synusium*, which develops very well in many forests in Japan, acts as a serious obstacle to the regeneration of young trees. The light condition below 3% prevailed on the soil surface more than 90% of the stand of *Fagus-Sasa*-association.

4. Discussion

In the foregoing chapters, we discussed the succession of plant communities on the basis of computation and illustration with very simplified community model and assumptions, and explained theoretically the relationship between a plant community and a succeeding one. When the plant community is vigorous and its leaf canopy is thick, the development of new succeeding community is

suppressed, but when the plant community is poor, or becomes over-matured, the development of the following community is somewhat easy. The clarified relationship, however, is based only upon the abstracted photosynthetic productivity and very simplified model—though the productivity is surely the most fundamental force for the development of communities—, so man may be afraid that these studies belong to a desk or deductive ecology, for we have shown no example for the succession of plant community in the field. Nominal series of successive plant community, which is generally described as one of the results of field survey, is pretended inductive, but in reality idealistic and deductive, because the nominal series is sometimes made up from comparative observations in a few days, without observations of the real succession in long time which is generally longer than a human life, and sometimes corresponds to a historical age. If the series of succession were observed on a definite site in a long time (e.g. in a few decades, see Braun-Blanquet, 1951, p. 435–438), the series would be only an occasional example of many developmental courses, for the progress of each succession of an association individual is always influenced and changed by affairs in chance. And the fragmental observations in the field bring us sometimes even complications, so some of the naturalists said that there was no succession series of the plant community, but merely the unpredictable change of plant species (e.g. Hayata 1929). After these opinions, we could only succeed to describe floristic composition of each plant community, but not find out the general law prevailing throughout plant communities. So we must study the vegetation on a fundamental basis. For the purpose, we need the theoretical analysis based upon the dry matter production, i.e. the most fundamental process of plant life.

In this paper, to our regret, the analysis is very superficial, as the result of the too abstracted plant community. We believe, however, that the critical problems in the succession process of plant community were fairly made clear in relation to light condition. The results obtained from this analysis may be very commonplace and very well-known facts. Without common theories, however, the isolated facts themselves, even if they are observed really in the field, remain descriptive ones, and there is no elucidation advancing step by step. The qualitative and isolated change in a population of plant species can generally be explained by this analysis as the causal result of quantitative and relative changes of metabolism. The scientific image of a concrete phenomenon must be built up as the reconstruction of more universal and more fundamental phenomena.

The analysis in the present paper may be able to explain clearly that the maintenance of thrived herb community continues very long time, but if the community somewhat degenerates, the invasion of young trees is very rapid, and after few decades and several years a young forest composed by invading trees can be established, as we see in Manaitabara, meadow on the foot of Mt. Yatugatake, Nagano Prefecture. There, the invading trees are mainly *Pinus densiflora*, somewhere *Betula Ermani* and *B. platyphylla* var. *japonica*, and *Larix leptolepis*.

Also in the subalpine region of Japan, evergreen coniferous forest, e.g. of *Abies*, *Tsuga*, *Picea*, develops as climax community, but among these tolerant

trees sun tree *Betula Ermani* almost always grows, so Nakano has settled *Betulion Ermani* as an alliance of the subalpine climax communities in Japan (1943, p. 188). This fact will be explained by the supposition of the fall of dominant shade trees in a group. Under the very wide opening, the light condition is better for the growth of the sun tree *Betula* than for that of the shade trees *Abies*, etc. When young closed birch forest has been established, the light condition under the new forest becomes worse for the seedlings of the sun tree itself and rather better for the seedlings of shade trees. A young low-layer of yearlings of shade trees develops under the canopy of birch. Too closed forest of sun trees kills the suppressed trees of the same species, consequently a number of sun trees disappear year after year, and there can remain only few dominant sun trees. Shade trees which grow under the birch canopy continue their growth, and some of them attain the height of the birches in due time. Hereafter, the growth of the shade trees becomes more rapid, and at last some of the sun trees surrounded by the vigorous shade trees dies in the shade of the latter. This succession series was observed at the subalpine forest (ca. 2300 m above sea-level) of Mt. Yatugatake, etc. This hypothetical explain will be supported very clearly with the theoretical analysis mentioned in the foregoing chapter.

The fall of trees in a group is very remarkable on Mt. Simagare, of which Japanese name means the trees die out together in stripes. The area where the dominant trees fell is seen in a distant view as the white horizontal stripes. The growth of *Abies Veitchii* in this place was reported by Okubo (1938, p. 662), and its growth curve is very similar as that illustrated theoretically in Fig. 12. After the long suppressed period, the trees recovered their growth since the falling of dominant trees. The trees already reached their maximum height in several ten years after the opening had been made.

In future, we must compute for the more advanced and accurate discussion of succession process the dry matter production of the plant community with many leaf layers (cf. Monsi and Saeki 1953 p. 47 ff. Kusanaga and Monsi 1954 p. 317 ff.), and stem and root, or the tree growth will have to be treated on the basis of the dry matter reproduction, although the computation is very complicate, and nowadays we have not yet enough materials. And the field research must be carried out to get the data on the deviation of light intensity under the leaf canopy, on the number of seeds and of seedlings, etc.

Summary

The succession process of plant community was theoretically discussed on the basis of computation of dry matter productivity of plant.

1. The productivity of plant in succeeding generation is decided mainly by the light condition which determined by the rankness of the plant community now dominating, and by the height difference between the young plant and dominant plants. With the productivity and normal growth curve of tree, a growth curve of young tree in the shade of herb community was drawn. These procedures demonstrated clearly that the height and density of herbs are essential

for the dry matter production and growth of tree seedling growing in herb community. And the fate of young plant varies with the very slight difference of density of herb community. The young tree which grows up more rapidly in height has more chances for succession than that of the smaller growth. The difference between sun and shade tree was also discussed.

2. Theoretical discussion on the destruction of herb community by growing trees was carried out by means of disk model, i.e. a model of tree crown. The productivity of herbs under growing tree crowns was computed by means of the combination of an assimilation curve and the light intensity under the disk, whose heights, numbers, and arrangements are various. When the trees densely aggregate, the destructive influence of trees upon the herb community becomes so severe, as herbs should be eliminated under the light condition inferior to their compensation point. However, when the trees grow isolated, the herb community is destructed only on the limited area under each tree crown, and on the vast area out of tree crown it continues to thrive as before. The physiognomy of this stage of vegetation is known as park landscape. Field survey was also carried out with respect to the deviation of light intensity in herb community, in order to make clear the probability of growing up of the seedlings germinated in herb community.

3. In order to elucidate the regeneration of young trees in the forest community, we calculated the plant productivity under an opening which broke out in the forest canopy as the result of fall of dominating trees. Here the relative height, i.e. height/radius, of the opening is decisive for the light condition on the forest floor. If the relative height is large, the light condition is so worse, that the plant must die out under the light condition lower than its compensation point. During the rapid growth phase of upper tree layer, the height difference between the dominating trees and seedlings becomes rapidly larger, and the opening becomes relatively smaller, so the seedlings which started to grow in the light enough for the assimilation under the opening, must die in this phase soon or late. The decrease of dimensions of the opening with the stretch of tree branches around the opening is also serious for the fate of young undergrowth. The life and growth, consequently the succession of young trees may be just successive under the matured, or more easily over-matured forest canopy. Computation of the productivity and construction of growth curve made it very clear that the shade tree could tolerate the worse light condition than the sun tree.

We oblige to express our thanks to Prof. K. Hogetsu of the Tokyo Metropolitan University for his kind advice to the present research.

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Anatomical and Morphological Studies of Japanese Species of the Ophioglossaceae I. Phyllomophore*

By

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(Received August 31, 1954)

The structures of stems and leaves of the Ophioglossaceae exhibiting various interesting characters have long been investigated both externally and internally by many morphologists. Especially, the particular character that the sterile segment and fertile spike are held by a common stalk was repeatedly investigated and discussed.

In 1904, Campbell, studying on the affinities of the Ophioglossaceae and Marsiliaceae, concluded that two adaxial bundles in the common stalk of the Ophioglossaceae belonged exclusively to the spike. Campbell (1911, 1921), Bass-Becking (1921), and Maheshwari and Singh (1934) maintained that the so-called stele of the rhizome of the Ophioglossaceae was composed of leaf traces and the vascular supply to roots and that the basal part of a frond was the base of the petiole or a part of a leaf. Chrysler (1910) demonstrated the lateral marginal origin of the fertile segment in this family, and in 1945, from the view point of the vascular system, he stated that the construction of the shoot of *Botrychium* might be explained by Zimmermann's telome theory (1930). Bower thought in his earlier paper (1926) that the fertile spike of *Ophioglossum* consisted of the fusion of a pair of pinnae, but his hypothesis was altered later (1935) in describing that it was one branch of an ancient dichotomy which took place perpendicular to the other division occurring in the leaf. Basing chiefly upon the vascular system of the leaf, Ogura (1937) described a solid construction of the leaf of the Coenopteridaceae, and added that this construction might also be applicable to the Ophioglossaceae in interpreting that the fertile spike was homologous to the two basal pinnae of a fern, and in his "Anatomie der Vegetationsorgane der Pteridophyten (1938)" he distinguished vascular system of this family into five types. In 1950, the present writer examined the vascular course in the so-called petiolar base of *Botrychium japonicum*, and came to a conclusion that this part should be regarded as an intermediate organ between the rhizome and the petiole, and it was termed "Phyllomophore".

The present investigation has been carried on to ascertain whether the conception "Phyllomophore" could be or not be applicable to the whole members of the family.

Materials and methods. The materials used in the present study are 15

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species belonging to *Botrychium*, *Helminthostachys* and *Ophioglossum*, and they are mostly native to Japan proper, while some species to the Ryukyu Islands. In all cases the materials were observed in chief of serial sections through the rhizome, the phyllomophore and sterile and fertile segments.

The species studied are listed below following the system by Clausen (1938).

I. *Botrychium*

A. Subgenus *Sceptridium*

- B. japonicum* Und. (Ô-hanawarabi)
- B. ternatum* Sw. (Huyuno-hanawarabi)
- B. nipponicum* Makino (Aka-hanawarabi)
- B. robustum* Und. (Yama-hanawarabi)

B. Subgenus *Eubotrychium*

- B. Lunaria* Sw. (Hime-hanawarabi)
- B. lanceolatum* Angst. (Miyama-hanawarabi)

C. Subgenus *Osmundopteris*

- B. virginianum* Sw. (Natsuno-hanawarabi)
- B. strictum* Und. (Nagahono-hanawarabi)

II. *Helminthostachys*

- H. zeylanica* Hook. (Miyakojima-hanawarabi)

III. *Ophioglossum*

A. Subgenus *Ophioderma*

- O. pendulum* L. (Koburan)

B. Subgenus *Euophioglossum*

- O. vulgatum* L. (Hanayasuri)
- O. reticulatum* L. (Hiroha-hanayasuri)
- O. littorale* Makino (Hama-hanayasuri)
- O. ellipticum* Hook. et Grev. (Ko-hanayasuri)
- O. pedunculatum* Desv. (Kohiroha-hanayasuri)

Materials were fixed in formalin-acetic-alcohol for 2 to 12 hours, and imbedded in paraffin after passing through a series of ethyl and buthyl alcohol following a normal procedure.

1. Description

A. *Botrychium* Subgn. *Sceptridium*

Perennial herbs, terrestrial; moderate size. Bud smooth, completely enclosed by the sheathing base of the phyllomophore. Sterile blades rather large, ternately decomposed, fleshy; ultimate divisions oblong, or lanceolate, prominently serrate. Spores mature mostly during September and October. Gametophytes somewhat flattened dorsiventrally; embryo with a suspensor and the primary root growing from the lower surface of the prothallium.

Botrychium japonicum Und. (Fig. 1, C, D)

The leaf trace* arises from the solenostele of the rhizome. It is slightly curved in cross section, and is composed of a group of tracheids arranged mostly in regular radial rows. The similar group of tracheids is found also in the stele of rhizome. At the base of phyllomophore, the leaf trace curves into a form of C in cross section. Buds consisted of five successive leaves are enclosed

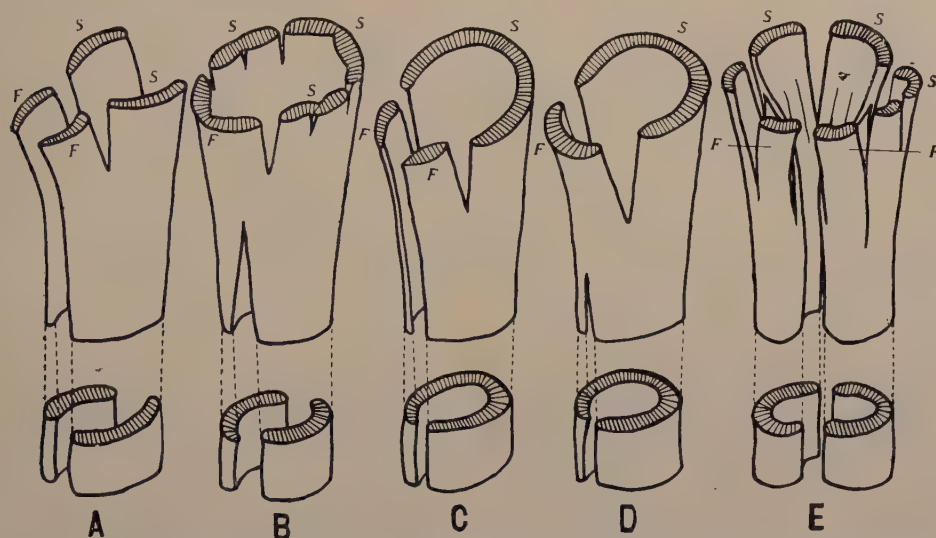


Fig. 1. Vascular system of top and middle part of phyllomophore: A, *Botrychium Lunaria*; B, *B. lanceolatum*; C, and D, *B. japonicum* (and *B. ternatum*); E, *B. virginianum*.

by its sheathing base, the leaf trace curves more and it is soon divided into two smaller bundles, each of which is provided with a single protoxylem. This division is perpendicular to the leaf trace departure, that is, in radial direction, though it is not always. They rotate gradually until they face one another with their xylem. Soon after, the bundles which enter into the fertile spike arise directly from the margins of the bundles. Preparatory to their division, these bundles increase considerably in size and are arranged in a form just like a pair of parenthesis, and the protoxylem of each bundle is also divided, and at the level slightly below where the fertile and sterile segments separate, each

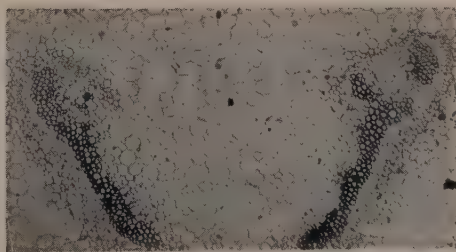


Fig. 2. *Botrychium japonicum*: sterile branch showing the departure of the trace for a lateral pinna in the extra-marginal manner. $\times 30$.

* The vascular bundle system of phyllomophore arising from the stele of the rhizome may be described as leaf trace, for convenience of description.

bundle is divided in the tangential plane into two of unequal size. This plane of division is parallel to that where the leaf trace leaves the stele of the rhizome, and is, naturally, perpendicular to the preceding division. After two bundles enter into the fertile segment, the remaining two run invariably through the sterile petiole until they reach the petiolar top where each of them issues extra-marginally a bundle for respective first pinna. Same cases are found in the fertile segment, and in this case, the vascular supply to the lateral pinnae sometimes occurs in the different levels.

***Botrychium ternatum* Sw. (Fig. 1, C, D)**

This species is characterized by both types of small and large sizes of the leaf and by extremely short phyllomophore.

The structure of the leaf trace is the same as in the former species, namely, it arises from the solenostele of the rhizome as a single slightly curved bundle provided with a single protoxylem, which is soon divided into two. A little higher up, it becomes to take a form of U and, then, closes into a continuous ring, though it is not always. Higher up, this ring is divided into two arcs of unequal size, and the smaller one enters into the fertile segment. In the latter the crescent bundle divides more or less equally, in a plane perpendicular to the preceding division. Each bundle is divided again into two, and soon small bundles are further separated from each median margin. Thus, at the top of the fertile segment usually six small bundles are arranged in an arc. Each bundle soon increases in its size. The marginal bundles supply for branches which eventually lead to sporangia.

On the other hand, the bundle in the petiolar base of sterile segment constricts at first at its median and then is divided into two. They run invariably through the petiole, until they are divided again at the top of the petiole, which are nearly the same in size. This division takes place in a tangential plane which is naturally perpendicular to the preceding division. Divisions, however, do not always occur on the same level.

***Botrychium nipponicum* Makino**

The vascular system in the leaf resembles also with the usual case of *B. japonicum*. So far as my present observation is concerned, both extremities of the curved bundle are separated and immediately after they are fused to form a bundle which soon lead to the fertile segment. The bundle which enters in the base of the fertile segment soon divides in the radial plane into two equal halves. They run invariably through the petiole and further separate the small traces extra-marginally at the petiolar top. This process is also repeated in the sterile segment.

***Botrychium robustum* Und.**

At the base of phyllomophore there is a slightly curved bundle similar to the case of *B. japonicum*. In the slender part of the phyllomophore it takes a form of C, which further closes into a ring just below the level where fertile and sterile segments are divided. Immediately after, this breaks into two unequal pieces lying in the radial plane. The smaller portion runs up into the fertile segment. The behavior of the bundle in fertile and sterile segments is

similar to that of *B. nipponicum* and *B. japonicum*.

B. Subgn. **Eubotrychium**

Perennial herbs, terrestrial; small size. Bud glabrous, completely enclosed by the sheathing base of the phyllomophore. Sterile blade pinnate or palmate, glabrous and fleshy; ultimate divisions oblong, ovate or deltoid. Spores mature mostly during July and August. Gametophytes small, somewhat flattened.

Botrychium Lunaria Sw. (Fig. 1, A)

The leaf trace starts from the rhizome in the form of a collateral endarch bundle with a single protoxylem, which splits very soon in a radial plane into two equal bundles, each provided with a single protoxylem. Soon they are twisted until they face with each other with their xylem. At the level where fertile and sterile segments separate, these bundles are divided respectively into two in a tangential plane. The members of a pair enter respectively into sterile and fertile segments. In the sterile segment, bundles again twist and are arranged side by side. These two bundles are subdivided into four arranged on a line, both bundles on the outer side entering into lateral pinnae. The process is also repeated in the fertile segment.

Botrychium lanceolatum Angst. (Fig. 1, B)

Behavior of bundle in the phyllomophore is similar to that of *B. Lunaria* excepting the mode of the branching of the bundles when the fertile and sterile segments are separated with each other. Near the top of phyllomophore, the bundles arranged in the form of a pair of parentheses undergo partial segregation and considerable increasement in size, and assume a form of ring. This ring soon splits, at first radially and then tangentially, to form four bundles. Two of them enter into fertile spike, while other two are again divided respectively into two and these run to three sterile segments. A little higher up each bundle at the base of the fertile spike is divided into two and further separates the small trace marginally at the petiolar top.

C. Subgn. **Osmundopteris**

Perennial herbs, terrestrial; large size. Bud hairy, partially covered by sheathing base of the phyllomophore, which is open on one side. Sterile blade large, deltoid, much divided, usually thin in texture, and sometimes hairy. Ultimate divisions ovate, oblong, or lanceolate, acutely or obtusely toothed or lobed. Spores mature mostly during June and August. Gametophytes short cylindrical.

Botrychium virginianum Sw. (Fig. 1, E)

As in the case of other species, a slightly curved bundle in the base of phyllomophore soon becomes C-shaped. As it passes through phyllomophore both ends curve to form inwardly curled ends. At the top of the basal sheath it is divided unequally in a radial plane. A little above, a rather small concentric bundle is separated from each median end. This condition is retained throughout nearly the whole length of phyllomophore, and at the level 1-2 cm below the point where the fertile and sterile segments separate, each adaxial side of two main bundles becomes hooked, and soon a small bundle is cut off

from its hooked end. Two small bundles thus formed migrate gradually to fuse respectively to the inner side of each main bundle. These bundles for the fertile spike are issued forth from adaxial portion of each main bundle in the extra-maginal manner. The two bundles which enter in the base of the sterile segment run invariably through the petiole, and further separate the pinna traces, which supply two lowest divisions of ternate sterile branch. The process is also repeated further in both sterile and fertile segments.

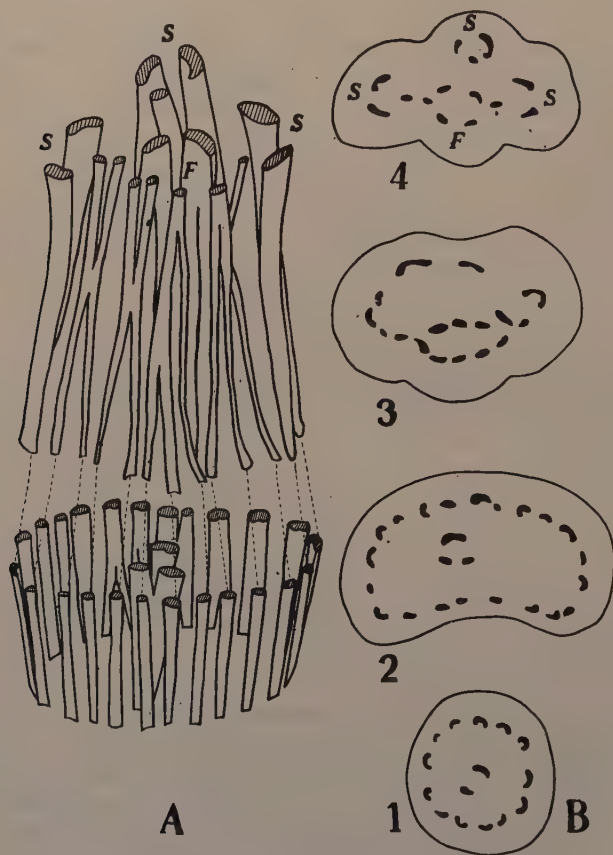


Fig. 3. *Helminthostachys zeylanica*: A, vascular system of top and middle parts of phyllomophore; B, cross section of phyllomophore; 1, the base; 2, the middle; 3, near the top; 4, the top. $\times 4$.

***Botrychium strictum* Und.**

The vascular system in the base of the phyllomophore markedly resembles with that of *B. virginianum*. Near the top the basal sheath, however, the bundle is not divided, but becomes a deeply curved arc with curled ends. Tracing the phyllomophore toward the upper portion, it becomes triangular and both arms elongate and are somewhat broadened. Near the top of the phyllomophore

the bundle closes itself into an O-shaped bundle enclosing a pair of small medullary bundles derived from both curled ends. Soon after these medullary bundles fuse respectively with an inner face of the main bundle. At a little below the level where the fertile and sterile segments are divided, the bundle is divided into four small bundles, three of them entering into the sterile segments, while another one, into the fertile. These bundles assume a form of C. The branching of bundles occurs in the same manner, excepting the fact that the bundle does not close into a continuous ring, but is issued off in extra-marginal manner as in *B. virginianum*.

D. *Helminthostachys*

Large perennials; rhizome creeping. Sterile blade palmately pinnate; venation dichotomous. Fertile segment spike-like, bearing numerous very short branches. Sporangia ovoid, each opening by a longitudinal slit. Gametophytes erect, and lobed below; embryo with a suspensor.

Helminthostachys zeylanica Hook. (Fig. 3)

The single trace arising from the rhizome is provided with a single group of the protoxylem. Then, it is divided into two mesarch bundles after the division of the protoxylem. Entering into the base of the phyllomophore, two bundles are divided into 12-20 bundles which are almost alike in size and are arranged in a circle, excepting two medullary bundles. During they run upward through the phyllomophore there occur some anastomoses between them, and the outer circle is transformed into a somewhat curved ellipse. On the other hand, two medullary bundles are divided into three or four small ones. Near the top of the phyllomophore the bundles approach one another and some of them fuse together and at the top of the phyllomophore, several bundles on the adaxial portion of the vascular circle form a smaller circle together with the medullary bundles, and enter into the fertile segment. The remainder of the bundles forms three groups of bundles which enter respectively into three lobes of the sterile segments.

E. *Ophioglossum* Subgn. *Ophioderma*

Epiphytic perennials; rhizome elongate, horizontal. Blade long strap-shaped, undivided or bifurcate towards the apex. One fertile spike borne medianly somewhat above the base on the face of the blade.

Ophioglossum pendulum L.

Three or four bundles are issued forth from the dictyostele of the rhizome. At the base of the phyllomophore, the leaf trace consists of from six to twelve bundles which are arranged in a circle. The number of the bundles is not always constant. The bundles in the cross section of the top of the phyllomophore, increase in number, and are arranged into two rows in the middle part, and into a row in the both sides. In the middle part the xylems in the bundles on both rows are face to face with each other. The vascular supply of the fertile segment is formed from four to seven bundles of adaxial side in the middle part. At the base of the stalk of fertile spike, the bundles are arranged in a

crescent, and they increase in number and ascend the stalk. On the other hand, the numerous bundles enter into the sterile segment and the vascular skeleton of the sterile blade is built up.

F. Subgn. **Euophioglossum**

Terrestrial perennials; small size. Blade simple. Fertile segment arising medianly from or below the base of the sterile blade.

Ophioglossum vulgatum L.

In the base of phyllomophore the leaf trace assumes the form of a slightly curved collateral endarch bundle with a single protoxylem group. The single trace is divided sooner or later into three bundles arranged on an arc. Two lateral bundles branch again and again and there are seven bundles arranged on a deeply curved arc at the higher part of the phyllomophore. A short distance below the branching level, both marginal bundles are divided again into two, and the bundles on the adaxial side, which are arranged on the chord, run into the fertile segment. On the other hand, the large median bundle of the arc is divided into two near the top of the phyllomophore, and the bundles on the abaxial side eventually run up to the sterile segment.

Ophioglossum reticulatum L.

The leaf trace arises from the solenostele of the rhizome as a slightly curved bundle. At the base of the phyllomophore, the bundles divided into three are arranged in an arc, though it is not always. A little higher up, one of the margins is again divided into two and arranged on the adaxial side, and then two others are also divided into two respectively. Thus, at the middle part of the phyllomophore, there are six bundles arranged on a circle. Slightly below the level where fertile and sterile segments separate, lateral bundles are divided respectively into two and then some other bundles are also divided into two. At the base of the fertile segment there are three bundles arranged in a crescent. Entering into the slender part of the stalk, the bundles are divided respectively into two and are arranged in a circle, which is maintained while they ascend a short distance below the level of sporangious region.

Ophioglossum littorale Makino

A single leaf trace is issued forth from the solenostele of the rhizome. As soon as it enters into the base of the phyllomophore, it is divided radially into three and are arranged in an arc, though their number is not constant. They are respectively subdivided into two at the middle part of the phyllomophore to form six bundles which are arranged in a circle. Three bundles on the adaxial side are almost alike in size. Higher up a little, bundles on the abaxial side are also divided to increase their number and are arranged in a deeply curved arc. Four bundles which enter into the fertile segment arrange themselves in an arc and their marginal bundles are again divided into two and these six bundles ascend the long stalk of fertile segment.

Ophioglossum ellipticum Hook. et Grev.

Similar with the other species belonging to the subgenus *Euophioglossum*, a

single leaf trace is issued forth from the stele of rhizome. At the base of phyllomophore, it is divided at first into a smaller and a larger ones, the latter of which is soon divided again into two. Three bundles thus formed are arranged on a crescent and run through the slender part of the phyllomophore. As they reach near the top of the phyllomophore each lateral bundle is subdivided into two. Just below the level where the fertile segment is separated, a pair of small bundles detach themselves marginally and enter into the fertile segment.

Ophioglossum pendunculatum Desv.

The vascular system in the base and the slender part of the phyllomophore is similar to that in *O. ellipticum*. Three bundles run through the phyllomophore until marginal bundles are subdivided near the top into two and migrate to the adaxial side, and three bundles of the adaxial side enter into the fertile segment. In the slender part of the fertile stalk five bundles are arranged in a circle, which is maintained while they ascend a short distance below the level of sporangogenous region. On the other hand, some bundles on the abaxial side are also divided to increase their number, and enter into the sterile segment.

2. Discussion

A. Vascular system in the phyllomophore. In many Ophioglossaceous species, a single leaf trace is issued forth as a sector of the solenostele, and after entering into the base of phyllomophore, usually it undergoes, sooner or later, some divisions and fusions which are particular in each species or each group. Hereupon, the presence of fertile and sterile segments causes special complexity of the vascular system.

In most species of the family, the leaf trace arises as a slightly curved bundle which is provided at first with secondary xylem. In this sector most tracheids are arranged in radial rows. The secondary structure which is prominent at the base disappears entirely in the slender part of the phyllomophore.

In most species of the family, the bundle in the base of phyllomophore increases, sooner or later, its number by division. It may rather be considered that this division has a close relationship with the telome-originated leaf. This extreme example is found in *B. Lunaria*. In this regard the subgenus *Sceptridium* is commonly obscure by the undivision of the bundle. If it may be that the apparent segmentation on the median part of the bundle at the base of phyllomophore of the subgenus *Sceptridium* represents an initial stage in the evolution of the branching method, the condition found in the most species of *Sceptridium* is primitive rather than derivative, while those of *B. Lunaria* belonging to *Eubotrychium* is derivative and most specialized. *B. japonicum* belonging to the subgenus *Sceptridium* seems to well illustrate the typical condition of the vascular system in this part.

In *B. ternatum*, also rarely in *B. japonicum*, however, the leaf trace remains undivided and it shows either a deeply curved arc or occasionally a continuous ring. In this case, a single trace which is issued tangentially forth from the stele, usually receives, in the phyllomophore, two important divisions, i.e., at

first in radial and then, in tangential, resulting a pair of small bundles for the fertile segment and a pair of large ones for the sterile. The first or the radial division, however, occurs sometimes so imperfectly or lacks entirely, showing a deeply curved arc or, more rarely, a continuous ring. The fact may appropriately be understood as a manifest repetition of the solenostelic character, that is, both the O-shaped bundle of phyllomophore and the solenostele of the rhizome are homologous and represent respectively a mesome.

In this regard, the subgenera *Eubotrychium*, *Osmundopteris* and *Euophioglossum* apparently belong to a similar category. In the subgenus *Osmundopteris* the slightly curved leaf trace at the base of phyllomophore changes later into C-shape as is found in *B. virginianum*, or takes the form of V as in the case of *B. strictum*. In most species belonging to the subgenus *Euophioglossum*, e.g., *O. vulgatum*, *O. littorale*, *O. reticulatum*, etc., the leaf trace arises from the solenostele of the rhizome as a single bundle and enters into the base of phyllomophore, and soon it is subdivided to form two or three bundles.

From the facts mentioned above, the vascular system in the phyllomophore of the Ophioglossaceae is characterized by that; 1) *B. Lunaria* and *B. virginianum* show a system similar to that of an ()-shaped bundle of normal case in *B. japonicum*, 2) *B. ternatum*, *B. nipponicum*, *B. robustum*, *B. lanceolatum* and all species belonging to *Euophioglossum* show a similar system to that of an O-shaped bundle of unusual case in *B. japonicum*; and 3) *B. ternatum* and *B. robustum* show a similar system to that of U-shaped bundle in *B. japonicum*. Thus, the vascular system in the base and slender part of the phyllomophore, in most members of *Ophioglossum*, and *Botrychium*, falls within the extents of variation represented by *B. japonicum*, though there are some differences in the level where the radial division occurs, if present.

In *Helminthostachys zeylanica* the bundles of the phyllomophore are arranged in a double ring. In the subgenus *Ophioderma*, e.g., *O. pendulum*, on the other hand, three or four strands are issued forth from a single gap of the stele of the rhizome and are arranged in a circle at the base of phyllomophore which is naturally more advanced.

Roeper (1826) suggested that in *B. Lunaria* the leaves arose in pair, one sterile and one fertile, with their petioles fused. Later, he improved his earlier suggestion that this was a part of a leaf which was regarded as the petiole. Braun (1839) thought that the stalk of the leaf was the confluence both of the fertile spike and sterile leaf. Zimmermann (1930) said that "Höchst eigentümlich den Ophioglossaceen ist die Verbindung des Sporangien- und Laubblatteiles: die ihren Achsen gemeinsame Verzweigungsebene steht nämlich senkrecht zur Verzweigungsebene der beiden einzelnen Teile." Chrysler (1945) mentioned that the aerial organ of the Ophioglossaceae should be regarded as a different form of the rhizome.

Most of these authors consider that this part is a fused organ. Recently, Clausen (1954) says that "Whether the fertile segments in the Ophioglossaceae be regarded as fused pinnae or part a dichotomous branching system, the result is the same. If the fertile and sterile segments are fused, the condition must be a specialization". Certainly, the fertile segment is a part of a dichotomous

branching system and homologous with the sterile segment. Indeed, in the vascular system of the phyllomophore in *Botrychium*, we can find no positive evidence of its fused organ and there we find one or more collateral bundles which extremely specialize in *Sceptridium*. Consequently, this part occurs itself as a fused form of the sterile and fertile segments, because of that, it is very difficult to distinguish clearly the difference of these two forms in a single bundle in *Sceptridium*.

On the other hand, if we receive Tansley's (1908) idea on the morphological equivalence of the vascular axis in leaf and stem of a fern, it can be easily considered that the shoot of the Ophioglossaceae consists of a congeries of branches. It may be also admitted Campbell's opinion who points out that "In the Ophioglossales, the stelar structure of the axis is built up exclusively of leaf-traces to which the roots are joined", and also "an absence of a cauline stele in the young sporophyte". Also, Bower says that "Where, as in the juvenile state of the Ophioglossaceae, the former (the shoot) is seen in its extreme state, there may appear to be no cauline stele".

That the leaf trace and the solenostele of the rhizome show, at a glance, different features is apparently due to the fact that the external form of the mesomes having the same origin takes different aspects in size and shape.

Judging from the branching manner of the leaf trace, it is clear that the leaf arises, as Ogura assumed, toward axis of the rhizome axially, and consequently the leaf is found to be an elongated organ holding cauline character.

A single leaf trace which is prevalent among the most species of the Ophioglossaceae is of course capable as a characteristic of primitive type from the view point of the comparative anatomy of the ferns, while its separation into distinct strands at the very base is a feature which is derived later.

In another side, in *B. lanceolatum* belonging to the subgenus *Eubotrychium*, the vascular course differs markedly from those of *B. Lunaria*. Namely, though the vascular system in the base of phyllomophore and the slender part is similar to that in *B. Lunaria*, it takes the form of O below the branching level of the fertile and sterile segments, and then the bundle splits into four parts in radial plane. Thus, this species is isolated from the category of the subgenus *Eubotrychium* and is rather similar to *Sceptridium*.

According to Nishida (1952), in the subgenus *Euophioglossum*, the second dichotomy of the bundle at the base of phyllomophore is also found. At its basal part of an adult plant the second dichotomy is not shown in spite of my careful observation, though its vestigial state may be found probably in the young stage of the *Euophioglossum* species. He reports that "It is difficult to follow them up as the anastomozation inserted between II and III. Apparently III occurs in a plane parallel to that of II". However, in my own observation it was found that the vascular system in the phyllomophore of species of *Euophioglossum* studied can rather easily be traced. I think it is important that there are two types in the formation of the vascular bundles leading to the fertile segment. In the first type the components of the adaxial bundle are prepared before the components for the sterile segment, while in the second type the components of the adaxial bundles are prepared after the component for the

sterile segment shows an arc-form arrangement. The former type is represented by *O. littorale* and *O. reticulatum*, while the latter is represented by *O. vulgatum* and *O. ellipticum*.

The leaf trace found in some species of the subgenera *Sceptridium* and *Eubotrychium* takes a solenostelic form below the branching level. This leaf trace may be formed as the result of the repetition of the form of a solenostelic bundle. The writer considers that the form is the most primitive and prior than that of the case of other species.

As already mentioned above, the phyllomophore and the stem are homologous and belong equally to the mesome. The writer can easily find that this may be interpreted as an occasional existence in the part of the rhizome accompanying difference in external form. The fertile branching in upper ends of the part is represented better than anything else in the state of this part. On the fertile branching it will be dealt with in detail later.

The vascular system in both sterile and fertile segments is rather similar to that of the condition in phyllomophore in the most cases. But, here we must pay a careful attention to the fact that the phyllomophore is the carrier of both sterile and fertile segments, both of which are homologous.

In a few species of the Ophioglossaceae the medullary bundles are observed in the phyllomophore. So far as writer's observation is concerned they are roughly divided into two different types in their manner of formation. In the first type or typical one, a medullary bundle is derived, at the base of phyllomophore from the bundles on the ring, *Helminthostachys zeylanica* being the only example of this type (cf. Fig. 2). After running a short distance toward upper part, it is divided into two, and at the vicinity of the top of phyllomophore they are subdivided respectively into two, situated on a crescent. These medullary bundles form, together with the bundles on the adaxial side of ring, two vascular circles, which enter, sooner or later, into the fertile segment. In the second type, the medullary bundle arises at the departure of the pinna trace in the top of phyllomophore as a provisional form. This condition is seen in connection with the extra-marginal method of the pinna trace, which is represented by *Osmundopteris* and *Sceptridium*, and this bundle has relation to the origin of the vascular supply of the fertile spike. Thus, the presence of medullary bundle has an important significance.

B. The vascular supply of the fertile segment. In most species belonging to the subgenus *Sceptridium* the bundles which supply the fertile segment arise directly from each edge of the C-shaped or parenthesis-like leaf trace. The typical example of this case is observed in *B. japonicum*.

In *B. ternatum*, the leaf trace in the form of a continuous ring is broken tangentially into two unequal portions, and smaller one, which may divide radially into two, runs up into the fertile segment on the adaxial side. The division of this case is performed in the plane parallel to that of departure of the leaf trace from the stele of the rhizome. Thus, these both divisions are comprehensible as the sympodial dichotomy. It is due to the assumption that the phyllomophore has a cauline character, and the phyllomophore and the rhizome are apparently homologous with each other.

On the other hand, radial division which is observed in the base of phyllomophore of most members of the family should be considered as provisional and of vestigial, because the bifacial structure in the base of phyllomophore is soon transformed into an unifacial structure at the top of the basal sheath, and in accordance with this transformation in its external feature, the bundle may change directly into U-shaped or ring bundle. Thus the writer considers that no importance should be laid on the radial division, though Chrysler (1945) maintained the morphological equivalence of all of these vascular branching.

It is indicated by the double vascular supply that the fertile segment of the phyllomophore in a certain number of species, e.g., *B. japonicum* and *B. Lunaria* corresponds to a fused organ of two branches which destinate marginally.

It should be noticed in this regard that the two traces for the fertile segment seem to behave independently, because they divide frequently, at somewhat different levels of the top of the phyllomophore.

Maekawa (1952) points out that the vascular system in the petiole of *Ginkgo* resembles that in *B. matricariaefolium* observed by Chrysler (1945). In his observation, the dichotomous divisions I-III occur so early within the short branch, in which each bundle between the leaf petiole and peduncle divides in the tangential plane into unequal parts, and the further process in each is formed as the result of repetition of true dichotomy. This fact seems to be also very important for the interpretation of the branching in primitive ferns. In all of the early vascular ferns which retain the most primitive and ancestral form, the fertile segment arises by unequal division in a plane perpendicular to that of the other divisions.

The family Ophioglossaceae is one indicating this characteristic feature, though in subgenus *Eubotrychium* the branching of the fertile segment is less apparent than in the case of *Sceptridium* on account of the subsequent dichotomies. This fact is, however, quite clear in that, the branching of the fertile segment cannot but occur because of the distal region (telome) possesses the regular dichotomous character. In this regard Chrysler (1945) says that "It appears then that in *Eubotrychium* the entire shoot may be interpreted in terms of telomes." I cannot agree with his interpretation, but think that both fertile and sterile segments of *B. Lunaria* are homologous and belong to the telome-originated leaf.

In the subgenus *Osmundopteris* a pair of vascular bundles which supply the fertile segment arise from near two edges of the adaxial side of U-shaped leaf trace. *B. virginianum* and *B. strictum* represent this type. The departure of this vascular supply is called extra-marginal by Bower. This condition roughly similar to that of pinna. From this fact, though both theories of Røper and Bower maintain that the fertile segment is homologous with two basal sterile pinnae, the fertile segment branches off at a level below the two basal pinnae. Consequently, strictly speaking, the fertile spike is not homologous to the two basal pinnae, but it seems to be homologous rather to sterile segment including all pinnae.

The branching of this case is sympodial and its plane is of course in the tangential. At all events, the branching of this fertile segment differs to other case.

In regard to marginal and extra-marginal origin of branch bundles, Bower (1923) says that "However unequal the division may appear, it may still be held as an unequally developed dichotomy. Such marginal origin of the pinna-trace is seen in *Aneimia* and *Loxsonia* and a good example is shown by *Pteris umbrosa*. This may be held to be the primitive method of supply of the lateral pinna, and it is characteristic of leaves of moderate size. But in many leaves of large size the meristele is strongly curved, and in these the origin of the pinna-trace is not by abstriction from the margin, but from a point or point on the abaxial or convex side of the curve. This has been described as "extra-marginal", and its simple example is seen in *Dryopteris vivipara*." From the Bower's opinion mentioned above, it is apparent that the extra-marginal type appears essentially as a consequence of large size and complication of the leaf, to which the curvature of the meristele is so closely related. It is probably certain that the extra-marginal manner has an intimate connection with the following fact that a small circular bundle meets with the larger one of the phyllomophore. The writer agrees to his opinion since the extra-marginal is found, in fact, only in such large-sized members of the family, as *B. virginianum* and *B. strictum*.

Bower (1891) holds an opinion that the position of the fertile segment is a ventral lobe of a leaf. On the contrary, Chrysler (1910) denies it and says that "If the organ is really ventral in origin, the vascular system ought to exhibit this feature, but it has been shown that in *Botrychium*, and probably in the other genera of the family, the origin of the vascular supply of the fertile spike is marginal or slightly away from the margin on the abaxial side. No examples of true ventral lobes have been adduced". It is true that a pair of vascular bundles which supply the fertile segment in most species of *Sceptridium*, *Eubotrychium*, and *Osmundopteris*, originate certainly from the two edges of the C, U or parenthesis-shaped leaf trace.

In *B. ternatum*, however, the supply to the fertile segment arises in the ventral side of the form of an O (Chrysler's (1945) pseudostele in *B. dissectum*).

Thus, the fertile segment in this species may be said both morphologically as well as anatomically as an organ which is truly ventral in origin. The writer considers, therefore, that the two cases mentioned above are equally present in this family.

The vascular supply of the fertile segment in *Helminthostachys* consists of four ventral bundles, two of them being derived from the medullary bundles and two from those of the ventral side of the peripheral ring. The departure mode of this case is one of the characteristic features in the genus.

In subgenus *Euophioglossum*, on the other hand, the bundles leading to the fertile segment do not diverge directly from lateral side but from the ventral. In this regard the writer's observation does not agree to Chrysler's (1910) view.

In subgenus *Ophioderma* the bundles leading to fertile segment is derived from on the ventral side of the leaves, but these bundles are formed at the base of phyllomophore simultaneously. Therefore, this mode of the departure quite differs from the other species. The writer wants to add here a short word on the origin of pinna trace. There are two types in the manner of the depar-

ture of the pinna traces. The first type, marginal of Bower, is represented by species belonging to *Eubotrychium* and *Helminthostachys*, while the second type, extra-marginal, is represented by many species belonging to *Sceptridium* and *Osmundopteris*. Commonly, in most cases, the pinna trace of the fern arises

Table 1.

Abbreviations and signs used are as follows:

Morphological features.

L: type of leaf. tp, ternately compound; pc, pinnately compound; pl, palmately compound; plo, palmately once compound; s, strap-shaped; su, single undivided.

f: surface of phyllomophore. U, unifacial (c, characterized by partial unifaciality); B, bifacial.

Anatomical features.

B: numbers of leaf trace on the base of phyllomophore.

F: type of the departure of bundles for the fertile spike and number of fertile bundles. L, lateral; V, ventral.

P: type of the departure of pinna trace. e, extra-marginal method; m, marginal-method; —, absent.

s: number of bundles for the sterile segment in branching place. 3g, three groups.

V: manner of arrangement of the bundles in the top of phyllomophore. (), parenthesis-shaped; U, U-shaped; O, continuous ring; Od, concentric non-continuous ring; Oe, ellipse; On, non-continuous ring; V, V-shaped.

M: median bundle in the phyllomophore. +, present; (+), provisionally present; —, absent.

S: stele of the rhizome. S, solenostele; S-D, solenostele or dictyostele; D, dictyostele; e, endarch; m, mesarch.

Plants	Morphological features		Anatomical features						
	L	f	B	F	P	s	V	M	S
<i>B. japonicum</i>	tp3	Uc	1	L2	e	1-2	(), U, O	—	Se
<i>B. ternatum</i>	tp3-4	Uc	1	V1-L2	e	1-2	O, U	—	Se
<i>B. nipponicum</i>	tp3	B	1	V1	e	1	O	—	Se
<i>B. robustum</i>	tp2	Uc	1	V1	e	1	O	—	Se
<i>B. Lunaria</i>	pc	U	1	L2	m	2	()	—	Se
<i>B. lanceolatum</i>	pl	Uc	1	L3	m	3g	O	—	Se
<i>B. virginianum</i>	tp4	B	1	L2	e	2	()	(+)	Se
<i>B. strictum</i>	tp3-4	B	1	L2	e	2	V	(+)	Se
<i>H. zeylanica</i>	plo	Uc	10-20	V4	m	3g	Od	+	Sm
<i>O. pendulum</i>	s	U	6-12	V4-7	—	17-25	Oe	—	De
<i>O. vulgatum</i>	su	Uc	1-3	V3-4	—	7-10	On	—	S-De
<i>O. reticulatum</i>	su	Uc	1-3	V3-4	—	8-12	On	—	S-De
<i>O. littorale</i>	su	Uc	1-3	V3-4	—	7-10	On	—	S-De
<i>O. ellipticum</i>	su	Uc	1	V3	—	5-9	On	—	S-De
<i>O. pendunculatum</i>	su	Uc	1	V3	—	5-9	On	—	S-De

sympodially from the parent bundle.

C. Systematic consideration. For the convenience sake of discussion on the systematic accounts of the family from the view point of the present study, some important external morphological as well as anatomical features are summarized in Table 1.

The Ophioglossaceae, a family retaining many primitive features, is composed of several genera which represent progressive stages in reducing series. As to the relationship between Ophioglossaceae and Coenopteridaceae, it was stated at first by Resault in 1875, and, later, Scott, Bower, Lang, Ogura and other investigators advanced their conception in such facts, such as the similarities in sporangial type, organization of the rhizome, and the relation of lateral members to the central axis. Certainly, this family is well interpreted as a relic of Palaeozoic flora.

The first genus *Botrychium* was established by Swartz (1800). Presl (1845) divided the genus *Botrychium* into two major subdivisions including 17 species. Prantl (1892) divided the genus into two subgenera, that is, *Eubotrychium* and *Phyllotrychium*, and Bitter (1902) agreed with him. Basing upon the presence of a suspensor, the penetration of the gametophyte by the primary root, etc., Lyon (1905) proposed the group of the ternate-leaved members as a new genus, *Sceptridium*, while Eames (1936) pointed out that these characters were scarcely sufficient to the warrant generic segregation. A little later, Clausen (1938) divided *Botrychium* into three subgenera, *Sceptridium*, *Eubotrychium* and *Osmundopteris*, saying that "Certainly, the gross morphological details of twelve species constituting *Sceptridium* do not afford a basis for generic segregation. Further, there are species in this subgenus which have characters tending towards *Eubotrychium* and other species with characters tending towards *Osmundopteris*. Sharp lines can not be drawn, therefore, between genera that may be segregated from *Botrychium*. It has consequently seemed better to retain the three major groups of species as subgenera". *Ophioglossum* and *Botrychium* were divided by Copeland (1947) respectively into three subgenera, i.e., the former into *Euophioglossum*, *Ophioderma* and *Cheiroglossa*, and the later. into *Eubotrychium*, *Phyllotrychium* and *Osmundopteris*.

From the foregoing descriptions and Table, it is evident that, at least from the view points of the vascular system, the subgenus *Sceptridium* differs markedly from the other subgenera.

Botrychium lanceolatum is different from the subgenus *Eubotrychium* in morphological and anatomical points, especially in vascular system in the top of the phyllomophore and palmately divided sterile blades. Consequently, it is natural and reasonable that this species should belong in the subgenus *Sceptridium*.

The second genus *Helminthostachys*, established in 1822 by Kaulfuss, includes a single tropical species from Indo-Malayan and Australian regions, i.e., *H. dulcis* which is synonymous to *H. zeylanica*. Bitter (1902) pointed out that it differed from other two genera in longitudinal split of the sporangia and also he agreed to Prantl's classification. Besides several features mentioned above, this genus is characterized by 1) a creeping rhizome, 2) palmately com-

pound frond, 3) free veins, 4) divided leaf traces, with a median one, 5) mesarch condition of the stele and marginal pinna traces. These are truly important characteristics of the genus, by which it is distinguished from other genera. Campbell (1911) said that "*Helminthostachys* is much the most aberrant of the Ophioglossaceae and in many respects shows a marked resemblance to the Marattiaceae" and Bower (1926) said also that "Though clearly a member of the family it stands as an isolated type". Moreover, Clausen (1938) pointed out that "*Botrychium* with open venation and relatively much divided leaf is occurrented the most primitive; *Helminthostachys* intermediate; *Ophioglossum* with reticulate venation and little divided leaf is most advanced". In *Helminthostachys*, the lateral veins run toward both sides from the midrib, nearly parallel with each other. Each vein is divided equal-dichotomously once, rarely twice, and they run without any connection each other, while the venation in *Botrychium* is a sympodial dichotomy which is a derivative of equal dichotomy. Thus, the veins of *Helminthostachys* is also different altogether from those of *Botrychium* and *Osmundopteris*. The writer is in an opinion, therefore, that this *Helminthostachys* should be transformed from the Ophioglossaceae to an independent family.

The third genus *Ophioglossum*, characterized by its reticulate venation and fertile spike, was established by Linnaeus in 1753 and was commonly regarded as typical of the family. Clausen (1938) enumerated 26 species in his Monograph, in which the genus has been subdivided into three subgenera, i.e., *Euophioglossum*, *Ophioderma* and *Cheiroglossa*, of which the first includes the majority of the species. These are mostly small in size and are provided with a simple sterile blade and an erect fertile segment. This subdivision of the genus, however, seems to me too minute when one considers slight differences of the external morphological, as well as the anatomical features among them. Numerous puzzling problems in the taxonomy of the genus are certainly due to such monotonous features of species, and Japanese species are also in the same case.

The subgenus *Ophioderma* established by Blume (1828) is characterized by origin and behavior of the leaf trace, epiphytic habit, structure of sterile blade and rhizome, etc., by which it may be easily distinguished from other subgenera. Presl (1845) recognized *Ophioderma* as the genus together with other five genera. However, other writers did not recognize his opinion. For example, Copeland (1947) said that "*Ophioderma* may not well be raised to generic status". This subgenus should, indeed, be regarded as the most advanced and specialized group of the family, and consequently, the writer should like to elevate this subgenus as the genus.

3. Résumé

(1) In the present paper 15 species of the Ophioglossaceae have been studied morphologically and anatomically, under the special purpose manifesting the true nature of the so-called petiolar base, which was termed as the phyllomophore by the writer in 1950 in his study on one species of *Botrychium*.

(2) Considering from the vascular system, the phyllomophore should ap-

parently be considered as a special organ showing a cauline nature which carries both the fertile and sterile segments, notwithstanding it has been considered by many previous authors as the petiole of a leaf or a common stalk of both segments.

(3) In most species examined, a single trace is issued forth from the rhizome, and at the base of phyllomophore it takes a form of slightly curved arc in cross section.

(4) In *Ophioglossum pendulum*, numerous leaf traces come from the rhizome and, at the base of phyllomophore, they are arranged on a circle, while in *Helminthostachys zeylanica*, a single trace is divided into numerous traces, which are at the base of phyllomophore arranged in two concentric circles.

(5) There are three main divisions in the vascular system, that is, 1) the departure of the leaf trace, in which a bundle is issued tangentially forth from the stele of rhizome, 2) the radial division at the base of phyllomophore, and 3) the tangential division at the top the phyllomophore, by which the bundles for the fertile and sterile segments are separated.

(6) The second or radial division occurs, however, sometimes so imperfectly or lacks entirely, and the bundles in the phyllomophore are U-shaped or O-shaped in some species of the subgenus *Sceptridium*, V-shaped in *Botrychium strictum*, circular in *Ophioglossum pendulum* and concentric circular in *Helminthostachys zeylanica*. Thus, the radial division is not important.

(7) The O-shaped bundle or circular arrangement of the bundles in the phyllomophore should be understood as the repetition of the solenosteric character of the rhizome. Such a consideration offers an apparent evidence that the phyllomophore may be cauline in its nature.

(8) The tangential divisions are found at the departure of trace as well as at the top of the phyllomophore, which are indicated by the diving planes between two organs of cauline character.

(9) The vascular supply to the fertile spike is "marginal" in most species belonging to *Eubotrychium* and *Sceptridium*, while in *Osmundopteris*, it is "extra-marginal". In *Euophioglossum*, *Ophioderma*, *Helminthostachys*, and rarely in *Sceptridium*, it originates from one side of the vascular ring or circle, though this is considered as a special case of the marginal type.

(10) In *Eubotrychium* and *Helminthostachys*, the supply for the pinna trace is marginal, while in *Sceptridium* and *Osmundopteris*, it is extra-marginal.

(11) Summarized the characteristics studied, the writer considers that the genus *Helminthostachys* should be transformed from the Ophioglossaceae to an independent family, the subgenus *Ophioderma* should be elevated to a genus, and *Botrychium lanceolatum* belonging to the subgenus *Eubotrychium* should be included in the subgenus *Sceptridium*.

In conclusion the writer wishes to express his hearty thanks to Professor Y. Ogura and Dr. S. Watari who gave kind advices and criticisms throughout the present study. The writer also wishes to thank Dr. H. Hara for his kindness of giving permission to use his literature. In the collection of material used in

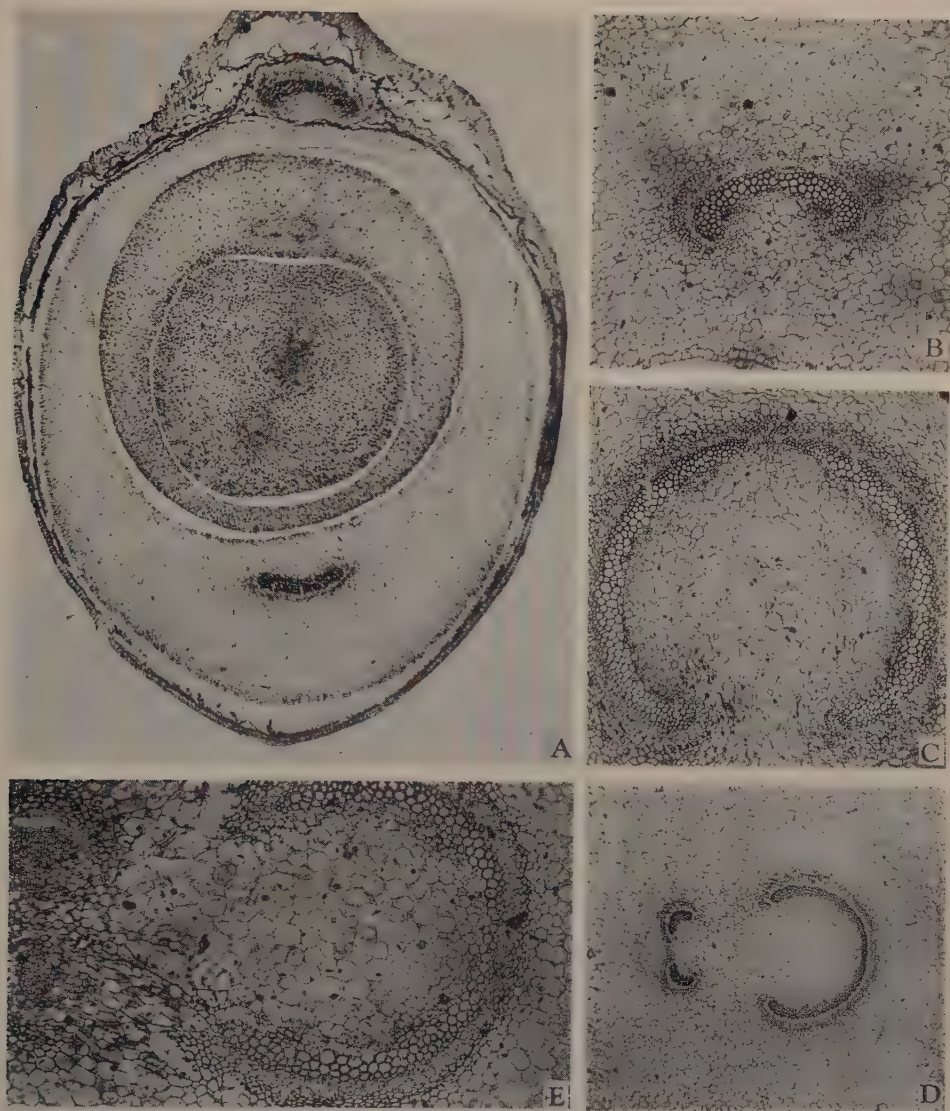
the present study, the writer's thanks are due to Profs. K. Hisauchi, K. Tanabe, H. Ito, M. Kasahara, T. Suzuki, S. Ono, E. Maeda, T. Ōmura and T. Namekata. Thanks are also due to Messrs. T. Tamayose and H. Kuroshima of Okinawa who kindly supplied valuable materials.

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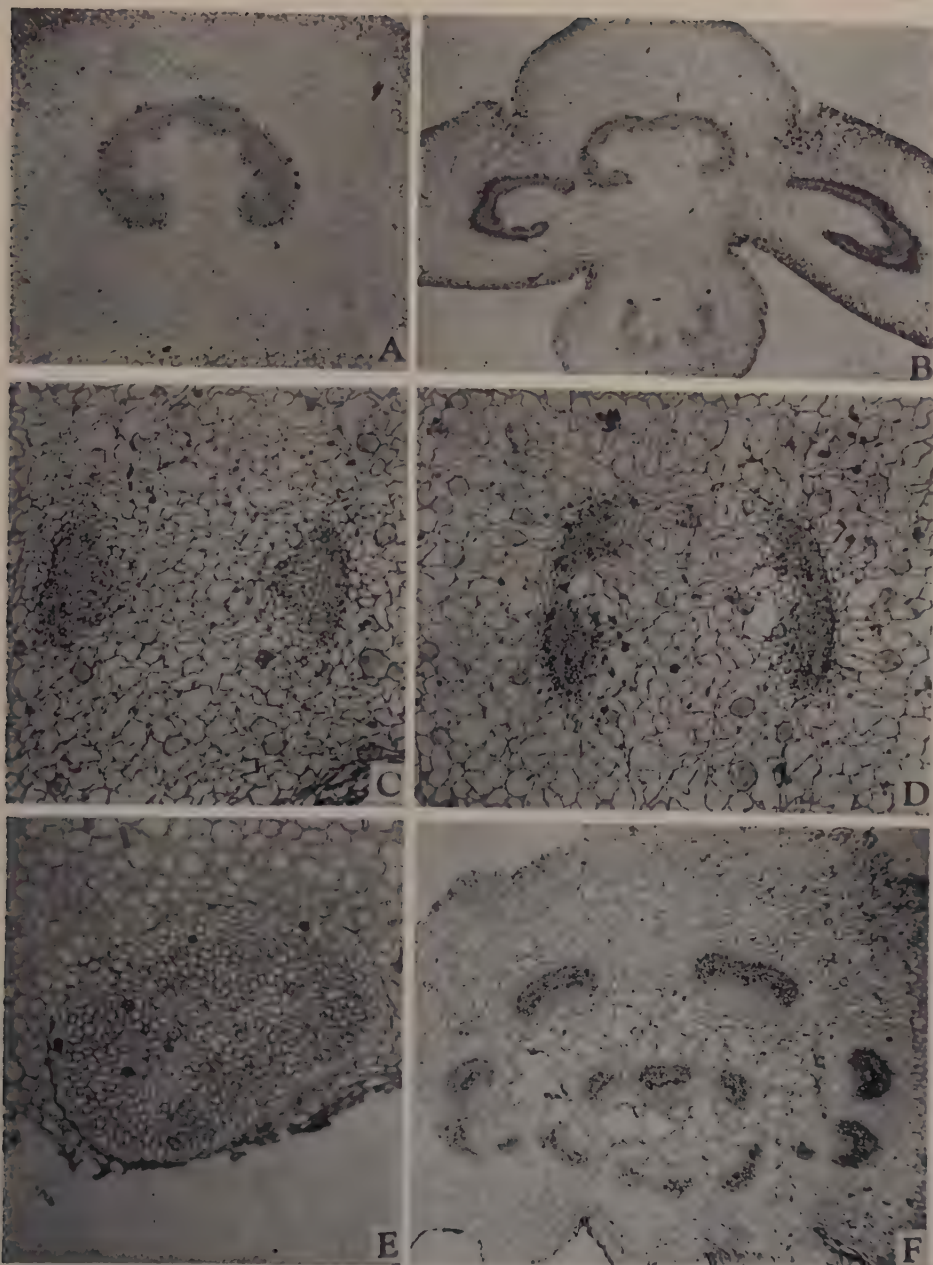
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Pl. I. A—D. *Botrychium japonicum*; A, sheathing base in which some buds are enclosed. $\times 20$; B, leaf trace at the base of phyllomophore. $\times 50$; C, ()-shaped bundle at the middle of phyllomophore. $\times 50$; D, extreme top, showing double vascular supply of the fertile segment. $\times 35$; E, *B. nipponicum*, showing double vascular supply of the fertile segment. $\times 50$.



Pl. II. A and B. *Botrychium virginianum*: A, base of phyllomophore. $\times 40$; B, extreme top of phyllomophore. $\times 40$; C and D. *B. Lunaria*: C, a little higher up of the base of phyllomophore. $\times 50$; D, near the top of phyllomophore. $\times 50$; E and F. *Helminthostachys zeylanica*: E, leaf trace in cortex of rhizome. $\times 50$; F, near the top of phyllomophore. $\times 40$.

Topo-morphological and Taxonomical Studies in Phaseoleae, Leguminosae

By

Fumio MAEKAWA

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1. Introduction

The bean family, Leguminosae, a large group with more than 13,000 species, spreading widely over the world, is clearly defined taxonomically by a single character—fruiting capsule in the special form called 'legumen' and seems to be a natural group, since it has been never confused with the other families. It has three under-groups, Mimosoideae, Caesalpinoideae and Psoideae usually as subfamilies, but often nowadays by some splitters as families, i.e. Mimosaceae, Caesalpiniaceae and Fabaceae. They are discriminated each other by their arrangement of floral part respectively, i.e. valvate tepals, imbricate petals in ascending sequence and the same ones following downward orientation. From the usual standing point of taxonomy which lays stress over the floral organization as itself, it may be said the treatment as a natural grouping, but under the concept of grasping every organ of plant body as the very part among the sequence of whole life cycle, this treatment seems to be unsound to say as natural classification. The flower organization must be reexamined under the scope of that idea, i.e., the flower is a part of the stem with leaf-complex in its elongation. And the author has reached to the conclusion that subfam. Mimosoideae is an amalgamation of two quite different groups, the one of which should be separated as subfam. Acacioideae.

For these ten years, the author has maintained the hypothesis of the leaf-class concept, under which only the relations between leaves and stems can be reasonably explained along with their evolutionary meanings. One of the important features in this concept, is to accept the simultaneous occurring of two or more kinds of different leaf-classes in one and the same shoot. Already in 1952, from these point of view, the author investigated the seedlings of some species belonging to trib. Phaseoleae and detected the organization of their primary leaves as a synthetic combination of two different kinds of leaf classes, i.e. between opposite SS leaf-class and opposite FF one in the manner of cruciate orientation.

The existence of a pair of primary leaves with simple lamina arranged in opposite phyllotaxis is widely distributed in the family. And, it is especially distinct in trib. Phaseoleae, as Wassiltschenko (1937) already pointed out its phylogenetic value in the bean family as well as with the evaluation of geophilous germination of seedling.

Concerning to the situation of stipules (S leaf-class under the author's concept)

of primary leaves, there are two distinct groups to be discriminated each other and moreover the detection of transitional example of disappearing of primitive characters in primary leaves in a genus of Phaseoleae, *Dumasia*, will be quite interest to explain the phylogenetic position of Phaseoleae among bean family. On the other hand, some variations, for example, whether sessile or petiolated in lamina of primary leaves, can be found in the taxonomical characters of them in Phaseoleae, and are, under the topomorphological concept, enough to demarcate some generic categories along with their differences in the presumable distributional center and external floral characters.

From the spring of 1951, through these three years, the author has joined with our colleagues in the investigations on "The growth and differentiation in higher plants" of which the financial supports have been mostly aided by the funds for scientific studies promoted by the Department of Education, to which he expresses his sincere thanks. In this study, *Phaseolus vulgaris* clon Master piece has been employed as the principal material for the investigations and its taxonomical studies have been destined to the charge of the author. Therefore, this report is one of the results obtained in that investigation.

2. Primary leaves (first foliage leaves) in Phaseoleae

Seedlings of bean family attracted already in early time the attention of some taxonomists and morphologists. De Candolle (1825) and Lubbock (1892) had illustrated some of the seedlings in their monographic studies. Later, Compton (1913) made excellent contributions to the vascular anatomy of the seedling of Leguminosae, but added a few to external morphology especially of primary leaves. Then, Wassiltschenko (1937) was the first examiner who evaluated the phylogenetic importances of the primary leaves in bean family. As to the primary leaves he pointed out the phyletic antiquities in 1) simple (not compound) lamina and 2) opposite (not alternate) phyllotaxis. He also enumerated as an ancient feature the geophilous germination of seedlings, which is not acceptable to the author. According to his conclusion, the appearing of antiquities above mentioned .e. simple laminas in opposite situation are widely and thoroughly distributed in both trib. Phaseoleae and trib. Dalbergieae.

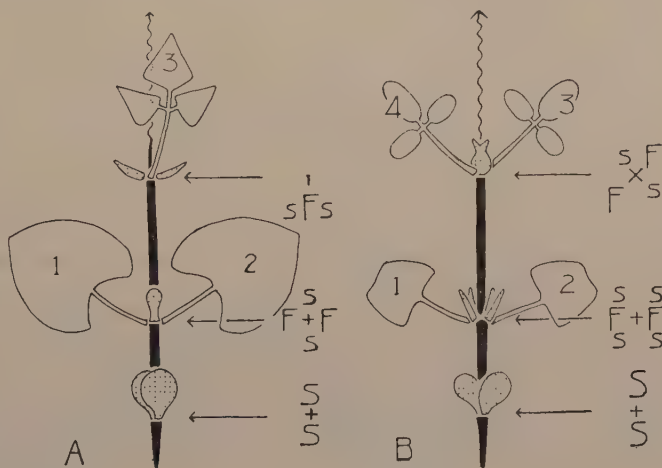
The following data in primary leaves of Trib. Phaseoleae are obtained by the author's investigations. The general plan of seedling is given diagrammatically in Fig. 1 A.

1) *Glycine Max.* Cotyledons epigeal in germination, obovately elliptic but slightly oblique in outline, thick in texture. Primary leaves opposite with very short petioles; lamina ovately elliptic to depressed orbicular in outline, apiculate with the tip of median costa, pubescent in undersurface. Stipules linear and 4 in number in two sets, to the both sides of the node of primary leaves.

Some varieties (for example, var. *Kurokurakakemame*, in which grain not globose but compressed in elliptic, testa dark deep green with a round area of bluish purple colouring along both sides of hilum) almost always have the second stair of opposite leaves, i.e. foliage leaves nos. 3 and 4, which are commonly in trifoliolate stage, have been combined into a set of opposite phyllotaxis.

(Figs. 1 B and 5) Rarely these two leaves spread along in one line, but are, having a short distance between the bases of them, not in a perfect opposite situation. The angles between this line of these two leaves crossed with cotyledonary line are 30° – 40° but not 90° , having a very suggestive meaning. Moreover, the stipules to these foliage leaves are often combined into nearly perfect integral or sometimes partly bifid one which means a very important significance. These remarkable phenomena explain directly the equifacies of primary leaves to their following ones and also the fact that the opposite phyllotaxis is ancient character to be followed by the alternate one of the other laminal leaves.

Fig. 1. Schema of seedlings. A. general pattern in Phaseoleae, B. *Glycine Max* var. *Kurokurakakemame*. Stippled area is S-class leaf and blank, F.



2) *Glycine Soja* (*G. ussuriensis*). The primary leaves, long ovate and smaller than those of *G. Max*. Stipules 4 in number, separated.

3) *Pueraria Thunbergiana*. Cotyledons epigeal oblong less than 1 cm in length. Primary leaves opposite, small, shortly petiolated. Lamina 8–12 mm long, depressed triangularly ovate, obtuse but apiculate in tip, almost truncate in its base. Stipules 4, lineari-subulate. The third and fourth, also fifth and sixth foliage leaves, set in each two to alternate in orixate pattern.

4) *Amphicarphaea Edgeworthii* var. *japonica*. Cotyledon hypogeal, shortly petiolated, with distinct cotyledonary bud in each axil which already elongated in the time of germination of the seeds. Primary leaves in opposite with long slender petioles, lamina depressed round-ovate, round and apiculate in tip, while nearly truncate in the base., dark greyish green and laxely pubescent, with lustrous and glabrate nebular areas along the costa and the basal part of the lateral veins. Stipules 2, interpetiolar, scariose and somewhat falcately lanceolate, rather large and broad as wide as the area between two primary leaves. (Fig. 4 D)

5) *Dumasia truncata*. (Fig. 4, A-C) Cotyledon hemisphaeric hypogeal but green in inner surface, remained in testa. Cotyledonary bud develops into a rather stout but long hypogeal rhizome. Primary leaves; petiole long, slender and translucent as like the stem; lamina rhombic triangular-ovate, mucronate

in tip, very broadly truncate at the base, yellowish green but distinctly purple in pulvinus. These two primary leaves are often in distance in alternated pattern, sometimes with the lower member much reduced in size, in extreme cases till to diminish to the scaly stipules without laminal F leaf-class. The stipules slender separate each other but tightly fastened to the stem as if intra-petiolar stipules.

6) *Canavalia gradiata*. Cotyledons large, erect, oblong, shortly attenuate in the base, rounded in the top. Primary leaves; petiole stout, shallow-grooved; lamina broadly ovate to nearly orbicular apiculate in tip, broadly and shallowly auriculato-cordate in the base, with the very short basal lobes which are somewhat acutish. Stipules 2, situated as interpetiolar style, obovato-spatulate, rounded in the tip, concave to inner surface. Epicotyl covered with rigid reflexed hairs.

7) *Dolichos Lablab*. (Figs. 2 and 6 D) Cotyledons epigeal. Cotyledonary buds often early developed with a simple leaf which grows in abaxial side of bud, long ovate in outline and has truncate base, with two stipules. Primary leaves

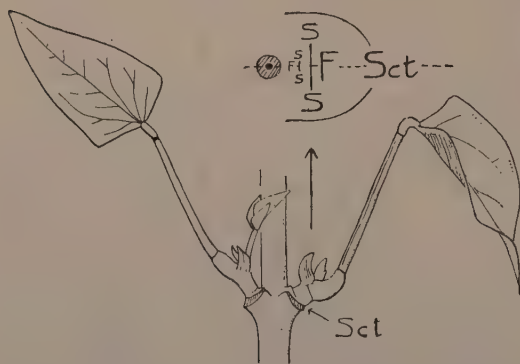


Fig. 2. Cotyledonary buds in *Dolichos Lablab*.

with stout petiole, rotundato-auriculate, round with very short acumen in the tip, distinctly auriculate in the base; bottom line of the sinus is straight and rectangular to the petiole. Stipules broadly lanceolate, acute, often 2-3-serrulate in the tip. Among the laminal leaves, nos. 3 and 4 and nos. 5 and 6 each respectively often combined in one set arranged in linear orientation and situated in orixate pattern. Nos. 3 and 4 often in opposite one.

8) *Rhynchosia acuminatifolia*. Cotyledon hypogeal. Primary leaves persistent till to autumn, opposite or often slightly dislocated along the stem and the lower one of the two, rudimentarily remained with stipules alone. Petioles long, divaricately stipuled in the base and in the tip, stipella distinctly reflexed.; lamina ovate, attenuate in tip with short acumen, truncately cordate in the base. Stipules 4, separate, broadly subulate, scaly.

9) *Stizolobium Hassjoo*. Cotyledon hypogeal. Primary leaves large and ovately hastate, basal lobes and basal sinus both in acute. Stipules separated.

10) *Rudua aurea*. (Figs. 3 A and 6 R) Cotyledon epigeal. Primary leaves opposite in sessile pattern. Lamina lanceolate, gradually acut to the top, roundly truncate in the base, erected in nyctinasty. Stipules 2, situated in the interpetiolar position, patently divaricate, very slender, subulate, fine in texture. Laminal leaves nos. 3 and 4 which are in the same plane, often situated nearly each other and crossed the line of the primary leaves with right angle. Stipules of these laminal leaves broadly ovate and much different in shape from those of primary leaves.

11) *Rudua Mungo* (Fig. 3 B). The seed brought back by the late Prof. T. Nakai from the Galang Isl. of Malay Archipelago on the year 1947. Cotyledons epigeal but the testa remained in the earth. Primary leaves are sessile, longly ovate-lanceolate somewhat attenuated to the tip, but slightly cordate at the base. Stipules oblong, situated interpetiolar space, emarginate in the top, broadly angustate to the base. The characters are very similar to *R. aurea* but differ in the laminal shape and broad stipules at the node of primary leaves.

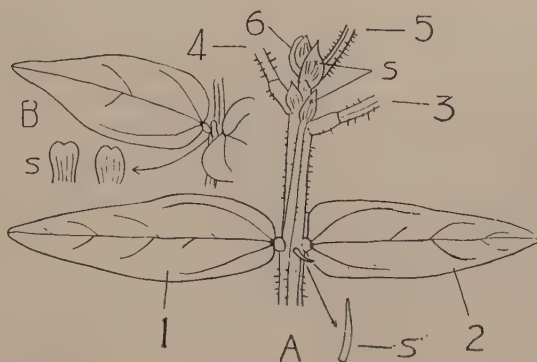


Fig. 3. Primary leaves in *Rudua aurea* (A) and *R. Mungo* (B). S. Stipule.

12) *Azukia angularis* (Fig. 6 A) Cotyledon hypogeal. Primary leaves longly petiolated. Lamina regularly ovato-cordate with short acumen in the tip, deeply cordate at the base, pendulous in nyctinasty. Stipules between two leaves, subulate, often irregularly bifid.

13) *Azukia Ricchardiana*. (Fig. 6 A) Cotyledon hypogeal. Primary leaves very elongated ovate, longly acuminate to the top, very shallowly but distinctly cordate at the base, pendulous in nyctinasty. Stipules similar as in *A. angularis*.

14) *Vigna sinensis*. (Fig. 6 V) Cotyledon epigeal. Primary leaves sessile in opposite pattern. Lamina ovate or ovato-elliptic, broadly acuminate to the tip but truncate at the base, lustrous and greyish green in the surface, erected in nyctinasty. Stipules 2, broadly subulate and situated at the interpetiolar area.

15) *Phaseolus vulgaris*. (Fig. 6 P) Cotyledon epigeal. Primary leaves very large, petiolated. Lamina broadly hastate but in some forms (Kentucky wonders, etc, broadly divaricated ovato-hastate) often undulated in margin, basal auricles and also margin of sinus both in round. Stipules broadly subulate subtruncate in the apex.

In the experiments when the seeds saturated with the vapour of methylester of 2, 4-D over 4 weeks or more, they germinate in all with metamorphosed primary leaves in which the lamina show much more thick structure and lost the auricle of the base, by the disturbance of mesophyll construction. Also there occurs, in the position of the leaf no. 3, a single-bladed foliage leaf, instead of a normal trifoliate one. Furthermore, the foliage leaves no. 4 and/or no. 5 often are brought to the multi-foliolate condition. These interesting modifications induced by the chemicals will be discussed by M. Furuya, the author's colleague in the investigations, in another paper in the same journal. Anyhow, it is remarkable that the characters, highly evaluated phylogenetically, can be modified by the chemicals.

16) *Phaseolus multiflorus*. Cotyledon hypogeal, large. Primary leaves similar to *Ph. vulgaris*. There can often be found the modifications to bring

out opposite pattern in the phyllotaxis between nos. 3 and 4 of normal trifoliate foliage leaves.

17) *Strophostyles*. (Fig. 6 S) This genus is native of North and Central America and the author could have no opportunity to examine them. According to Freeman (1913), tepary bean of Mexico and its surrounding areas, has primary leaves in opposite sessile pattern. Lamina triangulari-ovate, longly attenuate, subtruncate or more or less shallowly cordate at the base. Stipules are in interpetiolar situation.

The important characters in these materials are summarized in the following table 1.

3. Discussion

1) General considerations of evolutionary sequence in synthetic phyllotaxis.

As far as the investigations concerned, it is reasonable to abstract the common characteristics of trib. Phaseoleae as follows (Fig. 1 A):

a) simple primary leaves, b) one pair of the same, c) compound leaves in and upwards no. 3 laminal leaves, d) alternate arrangement in c), and e) small stipules at least two in the node of primary leaves.

Of course we may get some exceptions. Some genera have nos. 3-4 and nos. 5-6 respectively in combine as orixate pattern (*Pueraria*, *Dolichos*), even in the latter we see often even true opposite one in the set of nos. 3-4. While only one set of nos. 3-4 arranged in orixate pattern is in *Rudua aurea* and *Phaseolus multiflorus*, the latter may be changed to opposite condition. Among them, *Glycine Max* var. *Kurokurakakemame* (Fig. 1 B) has definitely opposite leaves in two stairs (one as primary leaves and the other, the set of nos. 3-4), being a bridge spanned between ancient opposite type to modern alternate one, and suggests that all the foliage leaves in alternate arrangement derived from opposite ones.

In 1952 the author investigated several genera of Phaseoleae (Fig. 1 A) and came to the conclusion that the leaves was a synthetic combination of opposite SS and opposite FF, i.e. cotyledons are cotyledonary phase in S-leaf-class (Sct) and two lamina of primary leaves are laminal phase of F-leaf-class (Fla) and their stipules must be considered as the diminute appearing of S-leaf-class in stipular phase, (Sst), so the set may be designated as (Sst Fla Sst Fla).

Concerning the combination of the two kinds of arrangement, the author has ventured to consider that the two combined in cruciate manner derived from spiro-scalate pattern to make up decussate phyllotaxis, by the reason of non existence of any other combined pattern except spiro-scalate one. Now, we may consider the fact again. In *Glycine Max* var. *Kurokurakakemame*, the two stairs cross in 30°-40° not 90°, contradictable to the usual acception. (Fig. 1 B). What means this fact? The author considers that this is the oblique crossing between F members and is the homologous phenomena of the one stair among spiro-scalate phyllotaxis in conifers, *Torreya*, *Cephalotaxus*, etc. (F. Maekawa, 1948) and in angiosperms, second pair of scales in axillary bud of *Zelkova* (F. Maekawa 1949) and *Fagus* (Furuya 1953). It may be explained as an older phyllotaxis, which is later emended to rectangular angles, perhaps in which the efficiencies

Table 1. Phylogenetically important characters in the leaves of some Phaseoleae

Genera or species	Cotyledons		Primary leaves				The other leaves	
	epigeal or hypogeal	opposite phyllotaxis	petiole	basal form	stipules in the node			
<i>Glycine</i>	epi.	+	short	round	4		+	1/2 phyllotaxis in the leaf no. 3 and following ones. The angle 90° between leaf no. 3 and the line composed of primary leaves
<i>G. Max</i> var. <i>Kurokura-kakemame</i>	epi.	+	short	round	4			nos. 3-4, opposite, and with 2 interpetiolar stipules; the angle, 30°-40°
<i>Pueraria</i>	epi.	+	long	truncate	4			+, nos. 3-4 and 5-6 often orixate
<i>Rhynchosia</i>	hyp.	+, often dislocation between two leaves, rarely lower one disappears	long	truncato-cordate	4		+	
<i>Stizolobium</i>	hyp.	+	long	hastate	4		+	
<i>Dumasia</i>	hyp.	+, often dislocation and disappearance of lower one	long	truncate	4		+	
<i>Amphicarpaea</i>	hyp.	+	long	truncate	2		+	
<i>Canavalia</i>	epi.	+	long	auriculato-cordate	2		+	
<i>Dolichos Lablab</i>	epi.	+	long	auriculate	2			+, nos. 3-4 often opposite. nos. 5-6 sometimes in orixate. First leaf of cotyledonary bud, in abaxial orientation
<i>Vigna</i>	epi.	+	— none	truncate	2		+	
<i>Rudua</i>	epi.	+	— none	truncate	2			+, nos. 3-4 often in orixate or nearly opposite
<i>Azucita</i>	hyp.	+	long	cordate	2		+	
<i>Strophostyles</i>	epi.	+	— none	truncato-acute	2(?)			+(?)
<i>Phaseolus</i>	epi.	+	long	auriculate, cuneate when treated with 2, 4-D-methyl ester	2			+, By 2, 4-D-methyl ester, no. 3 single-bladed, nos. 4 and 5 multifoliated.
<i>Ph. multiflorus</i>	hyp.	+	long	auriculate	2			+, nos. 3-4 often opposite or orixate.

of life phenomena must be highest (tab. 2, stage IV). While the nervation in cotyledon in spite of rather developed, is similar to that of stipules. And the stipules of primary leaves in *Phaseolus*, *Dolichos* etc. arranged with cotyledons in 2 rows as to concern with s-leaf-class. These arrangement is too curious to understand independently or under the concept of usual morphology, but when we take up theoretically the preceeding stage which becomes in later spiro-scalate one, it is quite acceptable as a more primitive arrangement of leaves, and now should be recognized the first step of opposite phyllotaxis as arrangement in two rows. These rows may be occur independently in S or F.

Table 2. Evolutional sequence of phyllotaxis established through the synthetic combination of S and F leaf-classes. The case of Trib. Phaseoleae, Leguminosae (F. Maekawa 1954)

Evolutional stages	Pattern of phyllotaxis	Remarks
I	$\begin{array}{ccc} S & S & S \\ & & \\ S & S & S \end{array} \rightarrow$	Opposite in two rows
II	$\begin{array}{ccccccc} S & S & S & S & S & S & S \\ & & & & & & \\ S & S & S & S & S & S & S \end{array} \begin{array}{ccc} F-F & F-F & F-F \\ S & S & S \end{array} \rightarrow$	Insertion of F; establishment of 4 rows with S and F
III	$\begin{array}{ccc} S & S & S \\ & & \\ S & S & S \end{array} \begin{array}{ccc} s & s & s \\ F-F & F-F & F-F \\ s & s & s \end{array} \rightarrow$	Complying of S to F
IV	$\begin{array}{ccc} S & S & S \\ & & \\ S & S & S \end{array} \begin{array}{ccc} s & F & F \\ F-F & s/s & s/s \\ s & F & F \end{array} \begin{array}{ccc} F & F & F \\ s/s & s/s & s/s \\ F & F & F \end{array} \rightarrow$	Spiro-scalate in F
V	$\begin{array}{ccc} S & S & F \\ & & s \\ S & S & F \end{array} \begin{array}{ccc} F-F & s/s & F-F \\ s & F & s \end{array} \rightarrow$	Deccusation in F
VI	$\begin{array}{ccc} S & s & F \\ & F-F & s/s \\ S & s & F \end{array} \begin{array}{ccc} sFs & sFs & sFs \end{array} \rightarrow$	Orixation in F, followed by alternation
VII-1	$\begin{array}{ccc} S & s & F \\ & F-F & s/s \\ S & s & F \end{array} \begin{array}{ccc} sFs & sFs & sFs \end{array} \rightarrow$	Establishment of one pair of primary leaves and alternation of F. VII-1 is the case of so-called 'connate stipules' and VII-2, with four stipules
VII-2	$\begin{array}{ccc} S & s & F \\ & F-F & s/s \\ S & s & F \end{array} \begin{array}{ccc} sFs & sFs & sFs \end{array} \rightarrow$	
VIII	$\begin{array}{ccc} S & sFs & sFs \\ & & \\ S & & \end{array} \rightarrow$	Lost of primary leaves, not yet found normally in Phaseoleae

Note: The division of small s is different category distinct from the evolution figured in this table and may be happened elsewhere after the time when the synthesis once occurred. In this table, it is treated as if occurred in stage VII.

The cotyledonary bud of *Dolichos Lablab* has the first leaf which situated in abaxial side of the axillary bud, against to main axis of the stem (Fig. 2).

This arrangement is very peculiar to the common understanding in current morphology, in which prophyll or prophylls always take place as in the cruciate orientation to the plane containing the axes both in main stem and axillary bud. But under the author's concept of leaf-class, it is clear that it shows the evidence of the insertion of F leaf-class to the primary two rows-arrangement of opposite phyllotaxis, in which two S leaf-classes in prophyll phase act, at the same time, as stipules inserted by the laminal leaf. It seems to be more proper expression, when we treat the inserted F leaf-class in axillary bud, as the one which has not yet get any firm connection in the arrangement between S leaf-class of main axis, but instead of it, there is more tight relation between both S members, i.e. cotyledon and so-called prophylls. It is sufficient enough to regard these phenomena as to the clear remaining of an establishment of a new monumental organization in that ancient day.

Supported by these two evidences for the evolutionary primitive characters, both the spiro-scalate pattern in foliage leaves nos. 3-4 of *Glycine Max* var. *Kurokurakakemame* and the start of SF combination in the axillary bud of *Dolichos*, the evolutionary sequences in the synthetic phyllotaxis of Phaseoleae, can be formulated as the following eight events or evolutionary stages (tab. 2).

- I) Stage in two rows.
- II) Stage of insertion of F to S.
- III) Stage of complying of S to F.
- IV) Stage of spiro-scalate in F.
- V) Stage of deccusation of F.
- VI) Stage of alternation through orixation in upper parts.
- VII) Stage of one pair of primary leaves.
- VIII) Stage of without primary leaves.

Thus *Glycine Max* var. *Kurokurakakemame* is in the stage VII checked by the restoration of short period of the stage IV. On the other hand, the main stem of *Dolichos Lablab* rests in stage VII and perhaps its somewhat unstable conditions may often induce it to go back to stage VI. While the cotyledonary bud of the same species has still retained the vestage of stage II. Almost all the members of the Phaseoleae are now staying in stage VII and yet some genera such as *Dumasia* (Fig. 4 A-C) and *Rhynchosia* are already protruding into the stage VIII.

The genera which still remain in the stage VII can be found not rarely in the bean family. Among them trib. Phaseoleae as well as trib. Dalbergieae are almost composed of the genera of the category, which explain that they are primitive in

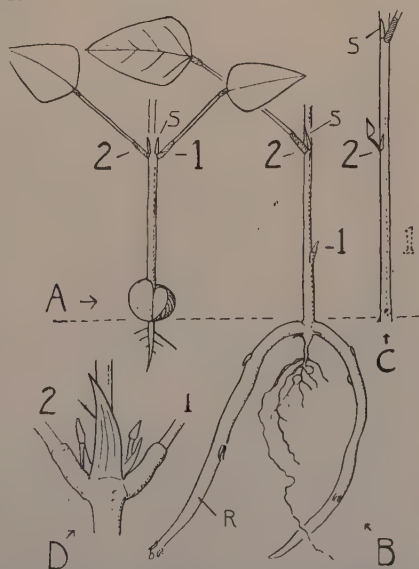


Fig. 4. A-C. Seedlings of *Dumasia*. D. First node in the seedling of *Amphicarpea*.

respect to the pattern of life cycles.

Table 3. List of bean plant in stage VII

Tribes	Genus or species	Remarks	Authors
Acacieae	<i>Acacia</i> sect. <i>Gemmiferae</i>		Wassiltschenko (1937)
Ingeae	<i>Albizzia</i>		W.
Adenantherae	<i>Pithecolobium</i>		W.
Piptadenieae	<i>Adenanthera</i>		W.
	<i>Entada</i>		W.
Amherstieae	<i>Hymenaea</i>		W.
	<i>Tamarindus</i>		W.
Eucaesalpinieae	<i>Caesalpinia</i> <i>Sappan</i>		Compton (1913)
Sophoreae	<i>Sophora</i>	simple leaves	C. W.
	<i>Maackia</i>	simple leaves	Hickel (1911)
	<i>Cladrastis</i>		Yanagita (1934)
	<i>Styphnolobium</i>		W.
	<i>Myroxyton</i>		W.
Podalyrieae	<i>Pultensea</i>		C.
Galegeae	<i>Psoralea</i>	simple leaves	W.
	<i>Amorpha</i>	simple leaves	H. W.
	<i>Indogofera</i>		W.
Hedysareae	<i>Desmodium</i>	simple leaves stipules 4	Kummer (1951), Maekawa
	<i>Lespedeza bicolor</i>	simple leaves stipules 4	Maekawa
	<i>L. cuneata</i>		
	<i>L. Davidi</i>		
	<i>Kummerowia</i>	stipules 2 simple leaves	Maekawa
Dalbergieae	almost all the genera		W.
Phaseoleae	almost all the genera	simple leaves	W. M. de Candolle (1825)
	<i>Clitoria</i>	simple leaves	Compton (1913)
	<i>Erythrina</i>	simple leaves	C.
	<i>Voandzeia</i>	simple leaves	C.
	<i>Kennedya</i>	simple leaves	C.

2) Stipules: connate versus lacerate

There are two categories in the stipules attached to the node of primary leaves. The first is the one so-called interpetiolar stipules, and can be found solitary in each internal area between the two petioles of primary leaves. Some of them are stout (*Canavalia*) but some, very delicate (*Rudua aurea* Fig. 3 A). The second one is two in set, in each side of the node. Thus the genera can be discriminated in two groups by the number of the stipules in the node of primary leaves. To the first group with two stipules, there belong *Amphicarphaea*, *Canavalia*, *Dolichos*, *Rudua*, *Azuki*, *Vigna*, *Phaseolus* and *Strophostyles*. While to the other which has four stipules, we can find the followings: *Glycine*, *Pueraria*, *Dumasia*, *Rhynchosia* and *Stizolobium*.

It is usual to consider the interpetiolar stipules as the one derived from the second condition which have four stipules in a node, through the fusing of two members in each set. According to this interpretation, there is no obstacle to accept 4-stipules as more primitive or, in other words, more ancient than two, i.e. interpetiolar stipules. This is the common way in the plant morphology. Nevertheless, already in 1952, had I ventured to insist the new interpretation in which the 2-stipules in vice versa can be traced back as more primitive one, from which, the 4-stipules derived by the laceration or splitting of preexisting one into two new members. The cases, *Pueraria*, *Turpinia*, *Adina* and *Kandelia* are enumerated and explained in the author's previous papers.

The decision whether is worthy in evolutionary tendencies of organ differentiation, is very much difficult and there are only scanty evidences. Fortunately the author can offer a good evidence for his interpretation; that is the case of the stipules to the leaves nos. 3-4 in *Glycine Max* var. *Kurokurakakemame*. It is a so-called connate one, but, if it really derived from separate 4-stipules through the amargamation in each other, the expected form of stipules in the induced node of leaves nos. 3-4, should be ancient form, similar to primary leaves, i.e. so-called separated stipules, as the result of parallel occurrence of older organization along with the similar older one, spiro-scalated opposite leaves (instead of more advanced alternate leaf in no. 3 in usual *Glycine Max*). The fact is not so, and the occurrence of advanced stage in stipules so-called connate one is much contradict with the ancient spiro-scalate form. It is much more natural to accept the stipules to the leaves nos. 3-4 which are recapitulated or restored pattern, induced along with the acceralation of oldest spiro-scalate pattern of phyllotaxis by some unknown acceralators. Furuya's experiments in *Phaseolus vulgaris* affected by the vapour of 2, 4-D methyl ester succeeded in finding some plants in which stipules often attracted, some times even in lacerated form, to the F leaf-class, removing from the ordinary situation to the margin of the petioles. From these evidences, it is much suggestible that this event of stipular laceration had, once in ancient time, happened to occur by the attraction of the neighbouring petioles of F leaf-class.

The first group, with more ancient 2-stipules is made up, as far as the author's materials concerned, with annual herbs. While the second group with the advanced 4-stipules is dominant with perennials, such as *Pueraria*, *Dumasia*, and *Rhynchosia*. The reason of this difference in the tendency is uncertain, but, in the author's view, it seems to be one of the evolutionary reasons, that the latter, having much longer periods of vegetative stage than the former, is favoured to receive in its naked vegetative cones with some modifications which

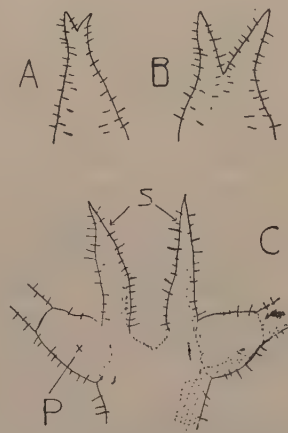


Fig. 5. Stipules of *Glycine Max* var. *Kurokurakakemame*. A and B. The ones to F_3 and F_4 . C. The same to primary leaves.

in some times, happen to become a new organization, such as for example, lacerated stipules. It is quite interest to find the same examples, in trib. Hedysareae in which *Lespedeza*, shrubby perennial genus, has four stipules in the node of primary leaves, while *Kummerowia*, the nearest akin to the former, but with annual habit, has two stipules instead of four. (tab. 3)

3) Sessile primary leaves.

Primary leaves in perfectly sessile form can be found in three genera, *Vigna*, *Strophostyles* and *Rudua*, the last of which a new genus proposed by the author already in the paper of 1952, but without no taxonomical diagnosis. The author gives here a diagnosis to it, to be valid and effective nomenclaturally, as follows:

Rudua F. Maekawa (Leguminosae, Trib. Phaseoleae) gen. nov.

Folia primaria binato-opposita, sessilia, nunquam basi auriculata, cum stipulis 2 ad nodo. Folia normalia trifoliata basi 2-stipulata. Flores in colore sulphurei; vexillum patentissimum basi profunde cordatum; carinae arcuato-falcatae sed nunquam spirales bullato-undulatae, in parte medianae gibbosae basi exappendiculatae. Stigma laterale; stylus stigmatem in appendice excurrente.

Typus generis. *Rudua aurea* (Roxburgh) F. Maekawa—*Phaseolus aureus* Roxb.

Etymologia nomiae generis: ex nomina chinense, ru-du (green bean).

Spp. *R. Mungo* (L. sub *Phaseolo*) F. Maek., etc.

It is near to *Azuki* Takahashi which has petioles in primary leaves and vexillum with nearly truncate base.

These three genera have their own distributional area in Eastern Africa, North and central America and tropical and subtropical Asia, respectively. (Ivanov, 1937).



Fig. 6. Primary leaves and stigmata of six genera, *Phaseolus* (P), *Strophostyles* (S), *Dolichos* (D), *Vigna* (V), *Azuki* (A) and *Rudua* (R).

While in these three areas, we can find the genera which are similar to the above mentioned one in their taxonomical characters respectively, especially the shape of stigma, the form and texture of petals. They are *Dolichos*, *Phaseolus* in strict sense and *Azuki*, and common only in having distinctly petiolated primary leaves. (fig. 6 and tab. 4)

These facts suspect the existence of three parallel evolutionary groups, i.e.

Phaseolus-Strophostyles, *Dolichos-Vigna* and *Azukia-Rudua*, instead of two groups, i.e. petiolate group of *Phaseolus-Dolichos-Azukia*, and sessile one of *Strophostyles-Vigna-Rudua*. The author inclines to accept that the sessile leaves at least in Phaseoleae, as an ancient character, while petiolated form is advanced one, inserted intercallarily with petiole.

Table 4. Parallel occurrence of six genera in Phaseoleae

Distributional area Primary leaves	America	Eastern Africa	Tropical and subtropical Asia
Petiolate	<i>Phaseolus</i> sensu strict.	<i>Dolichos</i>	<i>Azukia</i>
Sessile	<i>Strophostyles</i>	<i>Vigna</i>	<i>Rudua</i>
Stigma and tip of style	obliquely capitate	truncate, apical or lateral	perfectly lateral, appendaged
Flower colour	rose-purple dominant	variable	yellow dominant

4. Summary

The primary leaves of trib. Phaseoleae are described and discussed under the scope of author's leaf-class concept. (tab. 1)

In these tribes, Phaseoleae, Dalbergieae and Hedysareae, the evolutionary sequence of synthetic combination of S leaf-class and F one, both in opposite form, can be traced as in eight stages, which formulated in tab. 2.

In these sequences, the original starting point of 2-rowed stage of opposite leaves, the existence of spiro-scalate phyllotaxis in one variety of *Glycine* and the abaxial orientation of a leaf in the cotyledonary bud in *Dolichos Lablab*, are important contributions.

Connate stipules must be considered, at least in Leguminosae, Rubiaceae and Rhizophoraceae, as primitive stage, not induced one fused in two members. So-called liberated one, on the contrary, is to be accepted as the result of laceration in the former.

Sessile primary leaves shall be primitive, which may advance by the insertion of petiole. The evidences can be detected in six genera, from *Phaseolus* to *Rudua*.

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Effects of the Vapour of Methyl 2,4-dichlorophenoxyacetate on Growth and Differentiation in *Phaseolus vulgaris* L.

I. Formative Effects Induced in the Seedling after Various Grades of Application on Dry Seeds

By

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Introduction

Though the lower alkyl esters of 2,4-dichlorophenoxyacetic acid have been widely known as herbicides (Barron, 1951; Mullison, 1949; Mullison and Hummer, 1949), little attention has been given to the morphogenetical effect of treatments with these esters (Eames, 1949). In recent years the effects of the vapour of methyl 2,4-dichlorophenoxyacetate on growth and differentiation of the bean have been investigated in this laboratory. Thus, it is shown that each organ affected selectively by the stimulus of the chemical represents, as by the stimulus of other growth-regulating substances (Beal, 1951), a wide range of variety in the pattern of both gross external and histological responses. Not only tissues of different organs behave differently, but even within a same organ, the physiological age of each tissue at the time of treatment and the environmental conditions under which the tissue is grown play important roles in adding somethings to the chemically induced responses.

In this preliminary paper, it is described what behaviour of tissues and of organs has taken place in the seedling after the various grades of application with the vapour of methyl 2,4-dichlorophenoxyacetate on air-dry seeds of the bean. Then, further details in this work will be continuously published under the same title.

Material and Methods

Phaseolus vulgaris L., clone Master Piece, was used exclusively in this investigation. One hundred air-dry seeds of the bean harvested in the preceding autumn were treated with the vapour of methyl 2,4-dichlorophenoxyacetate in the same container as reported by Mullison and Hummer (1949); the container was placed in a dark room under constant temperature at 25°C. As the chemical is too insoluble in water to be employed in wide range of concentration in treatment, the seeds were exposed in the space saturated by the chemical for several long hours, i.e. for 1, 2, 3, 4, 5, and 7 weeks respectively. They were planted in pots filled with a mixture of sand, loam, and humus in the greenhouse until the fruits were fully ripened.

The preliminary experimentation had been made in the spring of 1951. And, the experimental results described in this paper were obtained mainly in May, June, and July, 1952, and supplementally in 1953 and 1954.

Experimentation

1. **Rate of germination.** The data for the rate of seeds germinating in 15 days after 1, 2, 3, 4, 5, and 7 weeks exposure to the vapour of methyl 2,4-dichlorophenoxyacetate are given in Table 1.

Table 1. Results of the rate of germination following treatment with methyl 2,4-dichlorophenoxyacetate for several long hours. Data are shown by the number of the seeds germinating (+) and not germinating (-); T, treated group; C, untreated control.

Period exposed (week)	1		2		3		4		5		7		Total
	T	C	T	C	T	C	T	C	T	C	T	C	
+	19	19	20	20	21	21	19	21	20	20	18	20	238
-	0	1	0	0	0	0	2	0	0	0	2	0	5
Total number of each group	19	20	20	20	21	21	21	21	20	20	20	20	243

$F=1.53$

$F_{0.05}=1.79$

Using the significance test (Snedecor, 1948), it was proved that the differences between the rate of germination of these above twelve groups are not significant, in other words, any influence can not be induced in the rate of germination after treatment with the chemical at least within seven weeks. The fact suggests that, in further studies, we need no consideration about the depression of germination following application of the chemical within seven weeks.

While in the percentages of seed-germination after one or two years exposure, we had 82% or 25% respectively. Judging from the fact that untreated controls germinated also about in the same percentages with those above mentioned, methyl 2,4-dichlorophenoxyacetate seems to have no effect for the rate of germination of the bean.

2. **Rapidity of development.** The delay in development was found generally in the seedlings of treated plants. Thus, to make clear the relation of the exposure period to the delay in germination, an attempt was made. In this experiment, times in days were measured that the plants need to grow to such developmental stage as shown in Fig. 1 in which the cotyledons just put forth from the ground (cotyledonous stage), and that, as in Fig. 2 in which the first foliage leaves are about to expand (first foliage leaved stage). The data for the delaying effects observed in these two stages are presented in Tables 2 and 3.

It is now clear that, both in the cotyledonous stage and in the first foliage leaved stage, the serious delay in germination is found in all of treated plants, since those differences in rapidity of development were significant at the five per cent point according to the *t* test (Snedecor, 1948).

Table 2. Delaying effect after treatment of methyl 2,4-dichlorophenoxyacetate observed in the cotyledonous stage.

Period exposed (week)		Number of samples	Days after planting to grow to the cotyledonous stage	Delay in development (day)	t test calculated	t _{0.05}
1	treated control	20 21	9.1 ± 0.8 8.5 ± 1.0	0.6	2.13	2.021
2	treated control	20 20	10.3 ± 1.2 8.7 ± 0.8	1.6	4.94	2.021
3	treated control	21 21	9.0 ± 1.1 6.9 ± 0.7	2.1	7.70	2.021
4	treated control	19 21	9.3 ± 1.3 6.9 ± 0.7	2.4	6.90	2.021
5	treated control	20 20	9.5 ± 0.9 6.6 ± 0.5	2.9	12.70	2.021
7	treated control	18 6	9.7 ± 1.5 6.6 ± 0.5	3.1	4.84	2.073

Table 3. Delaying effect after treatment of methyl 2,4-dichlorophenoxyacetate observed in the first foliage leaved stage.

Period exposed (week)		Number of samples	Days after planting to grow to the first foliage leaved stage	Delay in development (day)	t test calculated	t _{0.05}
1	treated control	18 20	10.9 ± 0.9 9.2 ± 0.9	1.7	5.73	2.704
2	treated control	20 20	11.5 ± 1.0 9.6 ± 0.9	1.9	6.75	2.704
3	treated control	22 21	11.0 ± 1.0 8.0 ± 0.9	3.0	9.83	2.704
4	treated control	18 21	11.2 ± 1.1 8.0 ± 0.9	3.2	9.65	2.704
5	treated control	20 20	11.4 ± 0.9 7.6 ± 0.7	3.8	14.85	2.704
7	treated control	18 7	10.9 ± 1.2 7.9 ± 0.4	3.0	6.10	2.807

The delay affected by the treatment of the chemical can not be compared each other quantitatively, because, as experiments have carried out in different season, untreated control plants have shown some differences in rapidity of development. Nevertheless, the tendency was found in general that the longer the period of treatment of the chemical, the more distinctive the inhibition of

growth was. On the contrary, though the statistical testing was omitted, time of the fall of cotyledons and of first leaves was considerably earlier in treated plants than in untreated controls. And, there was no delaying effect in and after the second trifoliolate foliage leaved stage.



Fig. 1. The cotyledonous stage of *Phaseolus*.



Fig. 2. The first foliage leaved stage of *Phaseolus*.

3. **Gross external responses of first foliage leaves.** One of the most obvious effects of the application of exposure to vapour of methyl 2,4-dichlorophenoxyacetate on dry seeds has been noticed in the morphological, both external and internal, responses of the first foliage leaves. Particularly, in plants pre-treated with the chemical for four weeks or more, the differentiation of mesophyllous cells was fully disturbed; instead of the formation of the palisade cells and the spongy mesophylls in mature leaf, the blade was filled with "replacement tissue (Watson, 1948)" that were separated by few or no intercellular space, having, in addition, the abnormal distribution of chloroplasts. This interesting result of anatomical modification and the comparison of it with the histological response of the grotesque leaves induced following treatment of 2,4-D



Fig. 3. The first foliage leaves showing the special feature of gross responses with application of methyl 2,4-dichlorophenoxyacetate. Left, treated plant; right, untreated control.

are, however, to be reported in detail in another paper (Hurusawa and Furuya, in press), and only the gross external responses will be presented in this chapter.

The treated first foliage leaf is illustrated by the photograph in Fig. 3. Resulting in a reduced lateral expansion of the interveinous tissue of the blade, lateral veins converge towards the midrib, and in consequence we can not find any auriculate basal form, specific to *Phaseolus*, in the lamina. Commonly, a dark ruffled margin, with closely packed chlorophyllaceous mesophyll, is induced by the marginal meristem, and is not found near the base of the blade. The zigzag form of the midrib is also resulted by unbalanced growth of the nerve and the mesophyllous tissues. It is remarkable that the vein eyelets distributing along the nerve are rich-green but the other are pale. And, no morphological effect is found both in the petiole of the first foliage leaves and in the pulvinus.

Further, to study of the degree of modification induced with treatment of various period exposure, the length of midrib, the size of lamina, and the length of petiole were measured in the mature leaves, and those results were described in Tables 4, 5, and 6 respectively.

Table 4. Length of mid-rib, exclusive of those of pulvinus, in first foliage leaves showing the severity of injury following treatment with methyl 2,4-dichlorophenoxyacetate.

Period exposed (week)	Number of samples	Length of mid-rib (cm.)	Ratio of treated to control	t test	
				t calculated	t _{0.01}
1 treated	33	4.7 ± 1.3	0.76	4.20	2.70
	23	6.2 ± 1.2			
2 treated	34	5.1 ± 1.3	0.76	6.24	2.66
	40	6.7 ± 0.3			
3 treated	31	4.5 ± 0.7	0.68	8.80	2.66
	33	6.6 ± 1.1			
4 treated	24	5.6 ± 0.6	0.85	3.88	2.66
	33	6.6 ± 1.1			
5 treated	41	5.1 ± 1.0	0.64	13.30	2.66
	37	8.0 ± 0.9			
7 treated	28	4.2 ± 0.9	0.67	7.08	2.70
	15	6.3 ± 0.6			

Inhibition of growth of the blade may be clearly recognized in every experimental group cited in Table 4, since differences in length of mid-rib were significant at the 1 per cent point according to the t test (Snedecor, 1948). However, when the zigzag mid-rib was stretched on straight, the length would be as long as that of the control. So that, it may be considered that the growth of mid-rib is not always inhibited at these experiments although that of the blade is so much inhibited.

Instead of difficulty in the direct measurement of the area of blades, the angle between both first lateral nerves was easily obtainable and better to explain

Table 5. Angle between both first lateral nerves of the first foliage leaves showing the reduction in size of lamina following treatment with methyl 2,4-dichlorophenoxyacetate.

Period exposed (week)		Number of samples	Angle between both first lateral nerves (degree)	Ratio of treated to control	t test t calculated	t _{0.01}
1	treated	30	47.5 ± 13.7	0.46	12.8	2.72
	control	25	98.6 ± 15.3			
2	treated	41	39.6 ± 13.8	0.41	15.2	2.66
	control	40	96.7 ± 18.9			
3	treated	34	39.0 ± 14.3	0.39	21.9	2.66
	control	35	99.0 ± 11.5			
4	treated	26	27.9 ± 10.8	0.28	23.8	2.66
	control	35	99.0 ± 11.5			
5	treated	39	29.5 ± 17.2	0.30	17.1	2.66
	control	38	97.7 ± 17.9			
7	treated	27	34.1 ± 13.4	0.33	15.6	2.72
	control	16	101.2 ± 10.9			

the result having been in proportion to the area of leaf.

At a glance, the reduction in size of lamina was distinctly observed in Table 5. By analysis of variance, too, it was indicated that the differences in angle were highly significant (odd=1:99). With respect to the ratio showing the

Table 6. Length of petiole of first foliage leaves showing the severity of inhibition with treatment of methyl 2,4-dichlorophenoxyacetate.

Period exposed (week)		Number of samples	Length of petiole (cm)	Ratio of treated to control	t test t calculated	t _{0.01}
1	treated	30	3.9 ± 1.1	0.85	2.37	2.70
	control	25	4.6 ± 1.1			
2	treated	35	4.0 ± 0.9	0.70	9.11	2.66
	control	40	5.7 ± 0.7			
3	treated	32	2.7 ± 0.6	0.54	14.90	2.66
	control	36	5.0 ± 0.6			
4	treated	28	3.1 ± 1.0	0.62	9.14	2.66
	control	36	5.0 ± 0.6			
5	treated	40	3.0 ± 0.8	0.52	14.30	2.66
	control	38	5.8 ± 0.8			
7	treated	28	3.5 ± 0.4	0.67	8.70	2.70
	control	16	5.2 ± 0.9			

reduction, those in length of lamina distribute about from $2/3$ to $3/4$ (in Table 4), and those in breadth about from $1/3$ to $1/2$ (in Table 5). These results show that the methyl 2,4-dichlorophenoxyacetate selectively affects inhibitory in the interveinous development of the mesophyll of first foliage leaves, but cannot block the development of the main nerve in first foliage leaves that has differentiated already in the seed-stage. In the relatively shorter period of treatment, occasionally even in the same blade, the mesophylls with normal organization are often formed in mosaic together with the veiny parts resulted by the inhibition of the growth and of the differentiation. However, the change of morphogenetic course arose perfectly and homogeneously in the first foliage leaf with application for the period of four weeks or more.

The elongation of petiole was generally inhibited with application of the chemical for two weeks or more, although a difference between the length of petiole in plants pre-treated for a week and that of untreated controls was not significant, but since differences between those of the others and the controls were significant at the 1 per cent point according to the *t* test respectively. However, no organographical and no histological response was found in this study.

4. Form and disposition of trifoliolate foliage leaves. The seedling of *Phaseolus* starts from a pair of opposite leaves, i.e. cotyledons (Fig. 4, C), and a pair of the first simple foliage leaves (Fig. 3, right; Fig. 4, a) that are abbreviated to F_{1R} and F_{1L} are produced in cruciate orientation to the cotyledons. Then, the first trifoliolate foliage leaf that is abbreviated to F_2 (Fig. 5) is formed on the third node in superposition of one of the cotyledons, and thus, the successive trifoliolate foliage leaves, that are abbreviated to F_3, F_4, \dots, F_n , respectively, are uniform in shape and are disposed in $1/2$ alternate system to the end of this development (Fig. 4, a). To the contrary, the seedling pre-treated with methyl 2,4-dichlorophenoxyacetate shows the organographically different form and disposition of leaves (F_2, F_3 , and F_4), but do not present such histological response as the inhibition of differentiation in the first foliage leaves above mentioned (in Chapter 3) and as the induction of conspicuous shoulder described in the following chapter. The changes occurring in number of leaflets and in phyllotaxis are eventually observed on the higher nodes than the third, and in results, there are many kind of patterns of the transition of form in the successive foliage leaves. These data obtained after treatment for various period were shown in Table 7.

By the results represented in Table 7, it was proved that methyl 2,4-dichlorophenoxyacetate affects to reduce the number of leaflets in the F_2 -leaf (that is to say, to induce the simple foliage leaf), and to increase the number of leaflets in the F_3 - and F_4 -leaf, and no response is always accepted in the F_5 - and the successive leaves (see, Fig. 4, b). That is, the inhibiting effects may be observed obviously in F_2 -leaf, though the severity of chemically induced modification seems to be less in the F_2 -leaf than in the F_1 -leaf. In the development of F_2 -leaf, methyl 2,4-dichlorophenoxyacetate had inhibiting effects on the division of growing point of leaf-primordium into three leaflets, but the chemical had no effect on the differentiation of mesophyllous tissue. Contrariwise, the promotive effects

to the growth of organ were always observed in the morphogenesis of the F_3 - and F_4 -leaves after treatment with the vapour of the chemical, and on this occasion, the chemical affected merely in the decision of the number of leaflets, but the shape and histological structure of each newly induced leaflet were just the same to those of the untreated controls.

It is evident that in the third node the formation of simple leaf might be induced with treatment of methyl 2,4-dichlorophenoxyacetate, since this meta-

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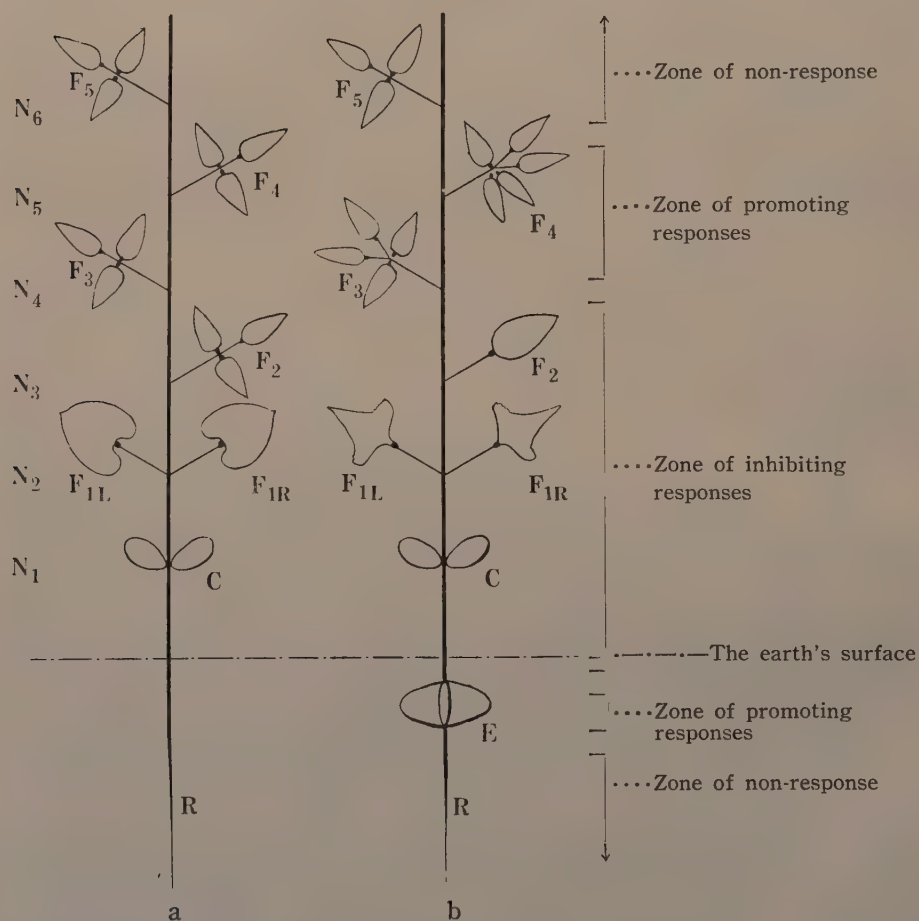


Fig. 4. Schemata of treated plant (b) and of untreated control (a) in *Phaseolus vulgaris*. C, cotyledon; F_{1R} and F_{1L} , first simple foliage leaves (primary leaves); F_2 , second foliage leaf, i.e. first trifoliolate foliage leaf in control; F_3 , third foliage leaf; F_4 , fourth foliage leaf; F_5 , fifth foliage leaf; E, abnormal structural enlargements induced in the transitional region between stem and root; N_1 , cotyledonary node, N_2 , second node, N_3 , third node, N_4 , fourth node, N_5 , fifth node, N_6 , sixth node; R, root.

Table 7. Patterns of form-transition and phyllotaxis in successive foliage leaves initiated on the third (N₃), fourth (N₄), fifth (N₅), and sixth nodes (N₆). Date are shown by the number of plants representing each patterns, and the numerals in the column of patterns show the number of leaflets in the leaf produced on each node. It is shown in the round brackets that the leaves formed experimentally on the same node. T, treated group; C, untreated control.

Pattern of successive leaves N ₃ N ₄ N ₅ N ₆	Period exposed (week)																Total	
	1		2		3		4		4*		5		5*		7			
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
F ₂ F ₃ F ₄ F ₅																		
1-3-3-3			1		1		2		1		5				5		15	0
1-3-(33)							1		1								2	0
1-(33)-3											2						2	0
1-3-4-3			2				1				3						6	0
(13)-4-3															1		1	0
1-3-5-3											1						1	0
1-(133)					1												1	0
1-(135)							1										1	0
1-4-3-3	1				2		1		4				1				9	0
1-(43)-3									1		1		2				4	0
1-4-4-3							1					1					2	0
1-(44)-3					1		2						1				4	0
1-4-5-3											1						1	0
1-5-3-3									5				4				9	0
1-(53)-3									1				1				2	0
1-5-4-3											1		2		1		4	0
1-(54)-3					1		1		1				2				5	0
1-(55)-3							1						2				1	0
1-5-(53)									1								1	0
1-5-5-3									1				1				2	0
1-5-6-3													1				1	0
1-4**							1		4				4				9	0
2-3-3-3			1		2						1						7	0
2-3-4-3			1												3		1	0
2-5-3-3							1		1								2	0
3-3-3-3	12	17	10	20	1	20	1	20	2	25		20		20	3	19	29	161
3-(33)-3															1		1	0
3-3-4-3			1	2													2	1
3-4-3-3				1		2					1						4	0
3-5-3-3	1						2										3	0
3-5-4-3	1					1											2	0
3-(54)-3						1											1	0
Total	15	18	18	20	13	20	14	20	25	25	17	20	19	20	14	19	135	162

* These experiments were carried out in May, 1954. But the other six groups were observed in May and June, 1952.

** That is the case in which the shoot apex is perfectly surrounded with the united petiole of two or more foliage leaves (see Figs. 18 and 19).

morphosis of F₂-leaf was observed in half of plants pre-treated for three weeks, in eighty per cent for four weeks, and in about ninety per cent for five weeks, but, to the contrary, in the untreated plants no metamorphosis was seen. There were not a few examples that the shape of F₂-leaf became perfectly to simple

leaf (Pl. III, Fig. 7), although some of them showed partially the remains of trifoliolate foliage leaves in the nervation or in the margin of blade (Pl. III, Fig. 8). These chemically induced simple F_2 -leaves, which could not be formed on the third node without treatment of the vapour of the chemical, seems to have the normal anatomical structure and physiological function, though there were many of the organographically abnormal examples in which the base of blade became to be conduplicatedly cordate. And, the bifoliolate foliage leaves which were induced by the stimulus of the chemical appeared occasionally only on the third node (Fig. 6 in Pl. III).

On the other hand, by the treatment with methyl 2,4-dichlorophenoxyacetate, multifoliolate foliage leaves were induced on the fourth and fifth nodes very often with the same frequency as the occurrence of the chemically induced simple leaf on the third node. The plants that produced multifoliolate foliage leaves on either the third or the fourth node, or on both these nodes, were seen about in twenty per cent of plants pre-treated for one week, forty per cent of them for two weeks, in the half of them for three weeks, and repeatedly in eighty per cent for four weeks. The cases in which the multifoliolate foliage leaves were formed both on the fourth and on the fifth node in the same plant, occurred less than half of the frequency of occurrence of multifoliolate foliage leaves in either of them.

Are these promoting responses occurring on the fourth and/or fifth nodes related to the inhibiting responses on the third node? In Table 8, it is found that the proportion of the occurrence of each pattern of transition in leaf-shape







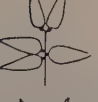
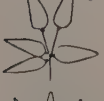


Table 8. Percentage of occurrence of each pattern of response of F_2 , F_3 , and F_4 -leaves pre-treated for several long hours.

Response		Period exposed						(week)
inhibiting (on the N_3)	promoting (on the N_4 & N_5)	1	2	3	4	5	7	
negative	negative	80%	55%	7%	7%	0%	21%	
negative	positive	13	17	31	5	3	7	
positive	negative	0	11	31	13	31	36	
positive	positive	7	17	31	75	66	36	

on the third, fourth, and fifth nodes in each experimental group were computed from the data cited in Table 7. Consequently, it was shown that the occurrence of promoting responses on the fourth and/or fifth nodes were more frequent than that of inhibiting ones on the third nodes in the experimental group pre-treated for one or two weeks, and that, on the contrary, the former were less frequent than the latter in the group pre-treated for four weeks or more. Because of insufficient details in these data, it was impossible to make clear the relation between these responses.

There are many kinds of patterns in the shape of multifoliolate foliage leaves, because the newly induced leaflets initiate from different portions of leaf-primordium. Table 9 shows the relative frequency of occurrence of each pattern

Table 9. Relative frequency of occurrence of each pattern in the shape of multi-foliolate foliage F₃- and F₄-leaves after treatment of methyl 2,4-dichlorophenoxyacetate. Degrees of occurrence are presented as follows: ‡ for most frequent, † for less frequent, + for least frequent, and blank for no occurrence. (See, the photographs of each pattern in Plates III and IV.)

Pattern of leaf shape	Period exposed						(week)
	1	2	3	4	5	7	
	‡	†	+	+	+	+	
	+	†	†	‡	‡	‡	
					+		
	+	+	+	†	†	†	
			+	+			
				‡	‡	‡	
					+		
	+	+	†	†	†	†	
						+	
					+		

in the multifoliolate foliage leaves produced on the fourth and fifth nodes by treatment for various periods. In the normal trifoliolate foliage leaves of *Phaseolus*, only the terminal leaflets has a petiolule with a pair of stipella, though the both lateral leaflets were directly connected to the petiole. Thus, the writers considered that, when two or more leaflets having their own petiolules were formed in a multifoliolate foliage leaf, the newly induced ones were originated from the meristem which has been fate to form terminal leaflet, but that, when a treated leaf produced three or more leaflets which connected directedly to the petiole without petiolule, these chemically induced ones were originated from those of lateral. It was found in this investigation that the occurrence of the chemically induced terminal leaflets was less frequent than that of lateral ones. It seems, however, that there is no difference in "readiness to response" to the stimulus between three growing points of leaflets in every primordium of trifoliolate leaf. Because, existing two growing points for lateral leaflets and another for terminal one in every primorium, the chance of response in the former is twice as that in the latter. And, as shown in Figs. 9 to 14 in Plates III and IV, the shape and size of leaflets were uniform and not modified in every multifoliolate leaf.

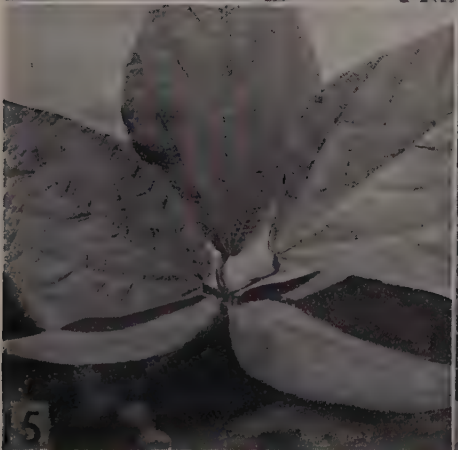
It was known that the mode of phyllotaxis changed in the seedling pre-treated with the chemical for three weeks or more. In this experiment, in the half of the plants which presented changes of phyllotaxis, the F_3 - and F_4 -leaves were concurrently formed on the fourth node, and about in ten per cent of those plants, the F_4 - and F_5 -leaves showed opposite system on the fifth node. And also, in ten per cent of those, F_3 -, F_4 -, and F_5 -leaves were simultaneously produced on the same fourth node. No opposite system has occurred both on the successive two nodes and on the higher than the sixth node. Even when on a node the opposite system or the verticillate system were produced, on the next node the phyllotaxis would revert to the original $1/2$ alternate system.

The curious phenomenon was observed on the fourth node in the plants pre-treated for four weeks or more, that the petioles of foliage leaves induced in the same node adhere each other to wrap up their shoot-apex. In these occasions, those which had no stipule and were formed as a tube because of the perfect adherence (Fig. 19) and those which had, because of imperfect adherence, a fissure on one side and only one stipule on the same side (Fig. 18) were seen nearly with same frequency between them. And, in the former no growth has been seen in both terminal and axillary buds of this fourth node, but in the latter the axillary bud starting from this node could continue to develop although the growth of the terminal one was perfectly inhibited (Fig. 18). Such grotesque compound leaf has, at least, more than six leaflets, and sometimes there was found the plants with a multifoliolate leaf that consists of more than twelve leaflets. Then, it is evident that such compound leaf seems to originate from two or more primordia of trifoliolate foliage leaf and are different from those which were formed from one leaf-primordium such as illustrated in Table 9.

5. **Behaviour of stipules.** The ontogeny of stipules and of stipella has been also affected morphogenetically with methyl 2,4-dichlorophenoxyacetate, though the writers do not touch the problems of these microphyllous leaves in



Pl. III. Several forms of the foliage leaves induced after treatment with the vapour of methyl 2,4-dichlorophenoxyacetate in *Phaseolus vulgaris*.—Fig. 5. Untreated controls of trifoliolate foliage leaf.—Fig. 6. Chemically induced bifoliolate F_2 -leaf.—Figs. 7 & 8. Chemically induced simple F_2 -leaf.—Figs. 9 & 10. Multifoliolate foliage leaves with four leaflets; the former has two petiolules, but the latter was one,



Pl. IV. Several forms of the foliage leaves induced after treatment with the vapour of methyl 2,4-dichlorophenoxyacetate in *Phaseolus vulgaris*.—Fig. 11. Chemically induced multifoliolate foliage leaf having two terminal leaflets but one petiolule.—Fig. 12. An example of fully divided petioles with two leaflets.—Fig. 13. An example of half-divided petiole.—Fig. 14. Multifoliolate foliage leaf with one petiolule and five leaflets.—Fig. 15. Multifoliolate foliage leaf with two petiolules and five leaflets.—Fig. 16. Foliage leaf with six leaflets and two petiolules.

the above chapters. However, it has been generally evident that the mode of formation of these microphylls was one of the species-specific characters that had been destined genetically and could not be affected with several environmental factors (Maekawa, 1952; Furuya, 1953). The constitution of lamina and stipules has not, hitherto, been variable naturally or under experimental condition.

In the first node of *Phaseolus*, cotyledons have congenitally no stipule and no stipellum. And, in the second node, there was the interesting fact that, in spite of the most severe modification in the blade of F_1 -leaf, no formative response took place in both stipella and stipules. In the third node, however, both stipules and stipella have responded remarkably to the stimulus, and represented the various degree of modification in shape and disposition. To the contrary, in the higher node than the fourth, the stipules did not take any change in shape and disposition, excepting the compound leaves resulted from the adherence of petioles, and also, stipella showed no response to the stimulus. Of course, in the chemically induced multifoliolate foliage leaves, if the newly produced leaflets have these own petiolules, i.e. if they are newly formed terminal ones, they present always stipella in normal shape and position similar to those of normal terminal ones.

17

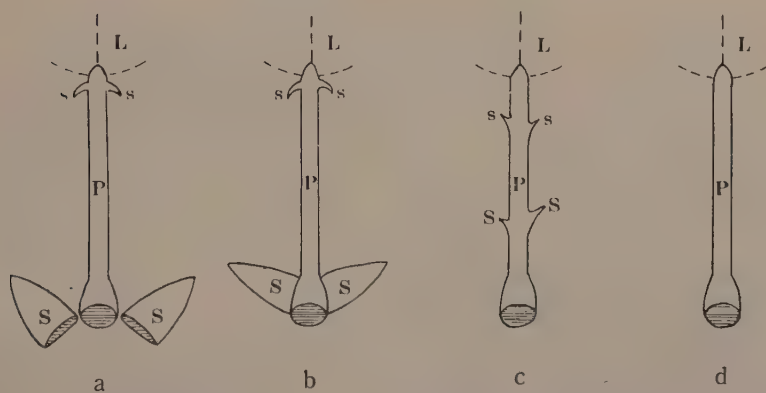


Fig. 17. Diagrammatic figures showing several situation of stipules and stipella. (a) untreated control of the bean; (b), (c), and (d) newly induced form. S, stipule; s, stipellum; P, petiole; L, lamina.

In the vegetative development of untreated beans, stipular leaves do not initiate from the petiole of foliage leaf but are produced directly on the stem (Fig. 20), and commonly, stipular leaves become mature earlier than foliage leaves in the same node. That is, the constitution of stipular and foliage leaves illustrated diagrammatically in Fig. 17-a, is regarded as "associated leaves in *metacomposed* shoot (Furuya, 1953)". However, in the treated plants with methyl 2,4-dichlorophenoxyacetate, this constitution of leaves has often changed in various ways. One third of plants pre-treated for four weeks or more showed the re-

duction of size only in the stipules of third node, but no change in the position was observed. And, in half of the rest, a pair of stipular leaves moved to the base of petiole from the stem (Fig. 21), and this new constitution may be called as "combined leaves in *pseudo-composed* shoot (Furuya, 1953)" (Fig. 17-b). Further, in some of treated plants, stipular leaves became smaller in shape and moved to upper position on the petiole, and stipella moved in concert to lower on the petiole (Fig. 22), and consequently, "simple leaf with stipella" illustrated in Fig. 17-c was formed experimentally. Finally, in extreme case, the formation of both stipules and stipella was fully inhibited, and then, the foliage leaves without stipule and stipella appeared alone on this node (Fig. 23, Fig. 17-d).

Thus, the behaviour of stipular leaves above mentioned has been observed both in the chemically induced simple F_2 -leaves and in the trifoliate F_2 -leaves of treated plants.

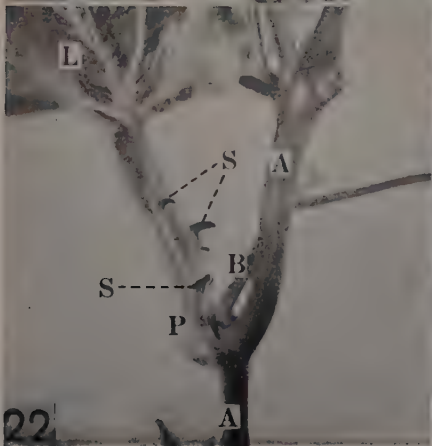
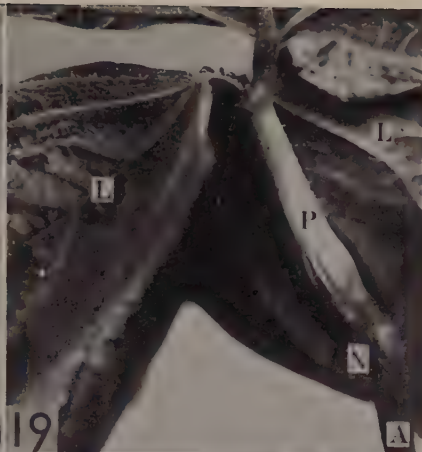
6. **Response occurred in the transitional region between stem and root.** Many formative effects were found in the aerial parts of the pre-treated bean plants, as described in above several chapters. While, in the underground parts were also observed conspicuous gross responses, which were the abnormal structural enlargements in the transitional region between stem and root.

At the third day after planting, in the seedling pre-treated for four weeks or more, a swelling might be visible in the region between hypocotyl and radicle, and, by the fifth day these newly initiated organs became almost mature and were shown as remarkable shoulders, whereas on normal plants the smallest lateral root began to develop (Fig. 24). By and by, being over such abnormal cell-proliferation and cell-enlargement, normal growth and differentiation took place in the underground system of treated bean. Therefore, the shoulders occurred in transitional region did not continue growing so long and could not become so large in size (Fig. 26). Sometimes, the board-root, i.e. 'Brettwurzel' by Boas (1949), composed of lateral roots which are closely placed in a row and perfectly fused in each other was formed, and grew to ten centimetres in length maximally. And, normal structural lateral roots have occasionally initiated vertically from the planes of mature shoulders.

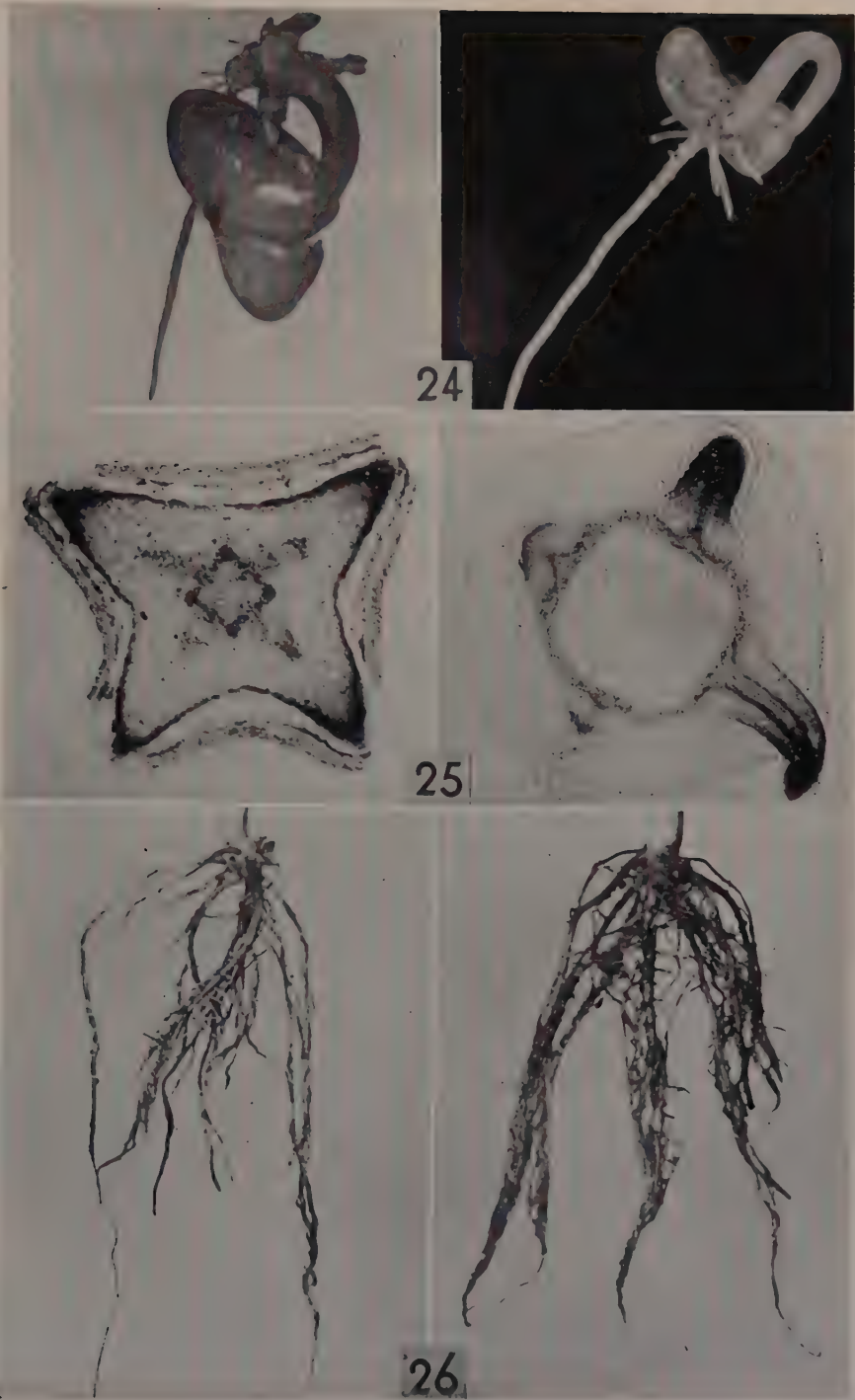
The anatomical modification of these broad, flat enlargements obtained in this experiment, was studied by S. Sato (Sato and Furuya, unpublished paper), and in consequence, it became clear that the anatomical structure of the newly induced enlargements in the transitional region between stem and root by treatment with methyl 2,4-dichlorophenoxyacetate (Fig. 25) was similar to those of the shoulders of tissue in the back of the root-tips after soil-treatment of 2,4-D (Wilde, 1951).

The number of the chemically induced shoulders was commonly four. However, other ones often formed in the space between these four shoulders, thus, from five to seven shoulders were seen in the same plant.

7. **Addenda.** Do various formative effects on the bean plant caused by methyl 2,4-dichlorophenoxyacetate bring forth only during the ontogeny? Or conversely, do some after-effects continue to the next generation? To make clear on this point, the following four groups were set up and the differences between them were examined in several characters. That is, this experiment



Pl. V. Fig. 18. The united petiole, which has a fissure on one side and only one stipule on the same side because of imperfect adherence.—Fig. 19. The perfectly united petioles, having no stipule and no bud.—Figs. 20–23. The various configuration of stipules and petiole; Fig. 20. The mature third node of untreated control; Fig. 21. This configuration shows the combined leaves; Fig. 22. The simple leaf with stipella; Fig. 23. The typical simple leaf without stipule and stipellum. A, axis of stem; N, node; B, axillary bud; S, stipule or stipella; P, petiole of the foliage leaf; L, blade of the foliage leaf.



Pl. VI. Abnormal structural enlargements in the transitional region between stem and root. Treated plants on left in each figure.—Fig. 24. Young seedlings, fifth days after planting.—Fig. 25.* Transverse section of this region, showing four conspicuous shoulders in treated plant, on the other hand, showing the formation of lateral roots in untreated control.—Fig. 26. Mature forms of the underground parts.

(* Concerning to these figures, we must express our thanks to Mr. S. Sato.)

was made extending over two years. In the spring of the first year, i.e. of 1951, the seeds pre-treated with the chemical for four weeks and the untreated control ones were separately planted, and in the summer the harvest was done in both groups respectively. Then, in the spring of the second year, i.e. of 1952, halves of the seeds taken from each group were severally treated again with the chemical for four weeks, and these two treated groups were grown in accompany with both non-treated another halves under usual condition. With regard to the four groups, rate of germination, rapidity of development, and changes in the mode of morphogenesis of each organ were investigated. In consequence, the clear results were obtained as shown in Table 10. In a word,

Table 10. After-effect test in the successive generations. +, plants present the changes in pattern of growth and differentiation; -, not responded plant.

Abbreviation of each experimental groups	Treatment with methyl 2,4-dichlorophenoxyacetate		Responses in the second year
	First Year	Second Year	
TT-group	treated	treated	+
TU-group	treated	untreated	-
UT-group	untreated	treated	+
UU-group	untreated	untreated	-

the chemical affects to only ontogenetic natures but not to genetic mechanism, because the difference between TT- and UT-groups and that between TU- and UU-groups were not significant, and contrariwise, TU- and UU-groups had significant difference to TT- and UT-groups. In other words, regardless of the preceding application with the chemical in parent-generation, the development of next one was affected by only the treatment in itself. It was clear that the responses induced after treatment with the chemical could not appear in the next generation and even when the treatment was made extending over three generations, no accumulative influence to the formative effects has been observed.

It was evident, although we have not studied exactly yet, that the seedlings pre-treated with the chemical for four weeks or more lack the abilities of phototropism and of geotropism in germination. That is, in treated plants, roots grew and stretched out from the earth, and on the other hand, shoots crept in the ground and could not grow normally. So, in this studies, we have always adjusted the abnormal features of them, and interestingly, the abilities for both kinds of tropisms can be recovered by the illumination of the sunlight.

Discussion

§ 1*.—Mullison and Hummer (1949) has demonstrated the depression of germination with the bean plants; among the seeds pre-treated with methyl 2,4-

* The number of the chapters in the discussion corresponds to that of experimentation in this paper.

dichlorophenoxyacetate for 30 days, 86% were germinating in 10 days (control, 98%), and those exposed for 60 days, 58% of which were germinating (control, 86%). However, we proved that the depression of germination does not occurred after the treatment with the vapour of methyl 2,4-dichlorophenoxyacetate within seven weeks in the bean seeds. We can see many reasons why such different results were obtained. In the first hand, there was the difference in the methods of application with the chemical or in the clones of bean. But, it seems the most powerful factor was the difference in the sorts of medium or that in the conditions during germination; that is, as reviewed by Crocker and Barton (1953), many of internal and external factors may be related to the germination of seeds. In our preliminary trials, it was known that the rate of germination showed difference in accordance with the sorts of medium as follows, i.e. wet filter paper, moist glass fibres, and moist sand or pot soil, and further, the different data were obtained between plants grown on the surface of these media and those planted in the ground. In this occasion, oxygen supply and moisture content probably play important rôle.

From the fact that the dormant seeds of subterranean clover, whether dry or wet, were resistant to 2,4-D, while seeds which had started to germinate were injured (Mitchell and Brown, 1947), it is suggested that methyl ester of 2,4-D does not also affect to the resting seeds of bean about the rate of germination.

§ 2.—In general, the acceleration of growth, i.e. increase of rapidity of development, seems to be a common property of growth substances, as worthy of this name, and the inhibition of growth may occur only at extremely high dilutions of these substances. Then, the application of the vapour of methyl 2,4-dichlorophenoxyacetate to the resting seeds of bean could compare to that at high dilution of such water-soluble growth hormones as IAA, 2,4-D, etc., since all of plants pre-treated with the vapour of methyl ester of 2,4-D has shown, more or less, delaying effect in germination (Tables 2, and 3).

In the studies of the effects of methyl 2,4-dichlorophenoxyacetate, it was found that, when the seeds were treated for relatively short period, i.e. probably for less than two months, the degree of delay in germination may be in proportion to the period of treatment (see, Table 2), and that, when the plants were, however, pre-treated for relatively long time, i.e. for a year or more, they did not present the delay which was expected theoretically from the results above mentioned. Therefore, the delay in germination induced by treatment seems to become into a constant value having no relation with the period exposed, and similar phenomena were seen often in other properties of treated plants.

Mullison and Hummer (1949) already reported the stunting of seedling and the slower germination of seeds following treatment with the chemical, but they did not show the quantitative investigation of the materials. Furthermore, they said that an interesting point about the delay in germination is that it often occurred initially in the germination process, and that, after germination started, the rate was frequently about the same as that of the control. According to our experiment of bean, it is agreeable that their latter explanation 'after germination started' must be corrected to 'after first foliage leaves stage of

seedling'.

And, the growth in length of all roots was remarkably inhibited in the early stage of germination, though the data was not cited in this paper. But, this inhibition soon disappeared (see, Figs. 24 and 26).

§ 3.—The terms 'inhibition of development' have commonly two different meanings; one of them is that the size of mature organ becomes smaller than that of controls, and another of them means the decrease in rapidity of growth, irrespective of the size of its mature form. Then, the examples of the former are reported in Tables 4, 5, and 6, whereas those of the latter in Tables 2 and 3.

Mullison and Hummer (1949) have found that the primary leaves of beans pre-treated with the vapour of methyl ester of 2,4-D presented typical malformation such as caused by 2,4-D when applied as a spray, and illustrated these injured leaves by the photographs of the seedlings. And, it was similarly shown in this studies that the modification of F_1 -leaves following treatment with methyl ester of 2,4-D are scarcely different from that of so-called grotesque leaves caused by 2,4-D. The common properties to them were as follows; organographically, the great reduction in size of the blade, the formation of thick, pale, and veiny ones, and the production of a darkgreen ruffled band around most of the blade margin (Eames, 1951); histologically, the formation of 'replacement tissue' which consists of rather thick-walled parenchyme-like cells that are turgid and are separated by few or no intercellular space, in lieu of the formation of normal chlorophyll-bearing mesophyll cells (Watson, 1948).

Further, according to the Watson's classification of severity of leaf injury, it is believed from our observation in this investigation that, in most of the plants pre-treated with the vapour of methyl ester of 2,4-D for one or two weeks, the severity of these F_1 -leaves was equal to that of 'category of 2: for less severe', and that, in the plants pre-treated for four weeks or more, it seemed to be 'category of 1: for most severe'.

And, from the photographs cited in both Eames' paper (1951) and Watson's (1948), the writers found clearly the zig-zag formation of mid-rib in injured leaves caused by the treatment of 2,4-D, so that, this phenomenon is thought as one of the common properties to 2,4-D and its methyl ester.

However, in spite of these common properties of the effects with 2,4-D and its methyl ester, it cannot be said that the both chemicals may always bring forth the essentially similar effects to plant tissue. Because, it was not, hitherto, proved that 2,4-D may cause such organographical modification as reported in Chapters 4 and 5. And, on the other hand, usually in comparison between effects caused by the treatment with the various growth-regulating substances, it must be remembered that the stage of growth at the time of treatment in the plant may decide the pattern of response in them. Dunham (1951) pointed out that rapidity of growth is an important factor in determining the susceptibility or tolerance to 2,4-D. And, it is impossible to compare the effect induced by treatment to resting dry seeds, as in this experiment, with that in the first foliage leaved stage of development, as in Watson's (1948). Namely, the comparison between effects by different chemicals have the meaning only on the base of the same stage of growth in plants at the time of treatment.

In general, the longer the period of treatment with the vapour of methyl ester of 2,4-D became, the more the degree of severity in response of plants increased, as shown in Tables 4, 5, and 6. But furthermore, when the period of treatment was lengthened, the degree of severity hardly enhanced in the proportion of the period of application, and showed a threshold in each property. In this point, it distinctly differs from the modes of the effects by the water-soluble growth-regulating substances.

§ 4.—In the so-called growth-regulating substances, it is characteristic that the activities of chemicals can be noticed in plants by the treatment with these very dilute solutions, and that, in reply both to the concentration of the chemicals and to the age of tissue at the time of treatment, they may affect not only inhibitorily but promotingly in a wide range to the growth and the differentiation of plants. But, when, in other chemicals, their effects to plants were represented only by inhibiting manner, even though the activities were observed in the extremely dilute aqueous solutions, the chemicals may be not named 'growth-regulating substance' but called 'poison'.

In previous chapters were discussed only the inhibiting effects of methyl ester of 2,4-D, and Mullison and Hummer (1949) also reported only the inhibiting effects of lower alkyl ester of 2,4-D in young treated plants and did not show any promoting effects of them. However, it was proved in this paper that methyl ester of 2,4-D could cause not only inhibiting effect but also promoting ones to the growth and the differentiation of bean plants. Therefore, this chemical is qualified for a growth-regulating substance on the definition above mentioned.

The rapidity of internodal growth of both hypocotyl and epicotyl was clearly inhibited by application of the chemical, though the anatomical structure of their parts were not affected at all. And, it is interesting that the first foliage leaves of treated bean showed remarkable inhibiting responses of laminal tissues, i.e. the formation of 'replacement tissue', but in the following successive foliage leaves, any histological change was not represented. On the other hand, F_2 -leaf was affected inhibitingly in the number of leaflets, while F_3 - and F_4 -leaves were caused the promoting effect, and in upper parts than those no response to the chemical was seen (see, Fig. 4). Then, from the fact that the organs of treated bean present each other different responses to same application with the chemical, we could be suggested that the difference of response coincide with the difference of maturity of each organ in resting seed-stage: the old organs, first foliage leaves, epicotyl, hypocotyl, and first trifoliolate foliage leaf, showed more or less inhibiting responses, while the younger organs, F_3 - and F_4 -leaves, and transitional region between stem and root, were affected promotingly, and finally, youngest parts were not responded at all.

What factors cause such opposed formative responses respectively to the tissues of bean plant though they are pre-treated with this chemical in the same condition? It is, however, impossible for the present to find the symptom coinciding with these various responses of successive leaves in the meristem of shoot-apex of pre-treated dormant seeds by means of anatomical and histochemical methods. Nevertheless, as regard to the maturities of each organ at the dormant

seeds of bean, the F_1 -leaves already developed sufficiently to be visible and took place the differentiation of tissue-elements, while the primordia of F_2 , F_3 , and the following successive foliage leaves were not yet initiated from the growth-point of stem-tip. Probably, in the treated beans, the varying patterns of response above mentioned may be resulted from the difference in the maturity of those tissues, or organs, at the time of treatment. Watson (1948) also found that the severity of injury increases in intensity from the next leaf above the normal leaf at the base of the stem upward to a point of greatest severity and from this point decreases in intensity toward the top of the stem where normal leaves are produced, and that the variety of pattern of response depends upon the age of bud at the time of treatment.

Even in the exhaustive experiment made by Watson (1948), the treatment of 2,4-D to the seedling of bean could not take place the chemically induced simple or multifoliolate foliage leaves in place of trifoliolate foliage leaves. On this property, it is evident that methyl ester of 2,4-D has the different action to bean plant from that of 2,4-D. But, it is overhasty to decide that 2,4-D has no such ability as its methyl ester has played, because, though it is necessary to put the materials in the same condition in order to compare the effects induced by these chemicals, the one was treated at the first foliage leaved stage of seedling while the other was pre-treated in the stage of dormant seeds.

In the treated beans, the opposite phyllotaxis occurred frequently on the fourth and the fifth node of seedling, in lieu of 1/2 alternate system. It is necessary to clear in these changes of phyllotaxis, that two successive primordia of foliage leaves are initiated simultaneously from the meristem of shoot-apex, or that, even if two successive ones are initiated one after another, the growth of internode between them is perfectly inhibited during the development. Then, it is thought from this result that native growth hormones may, more or less, concern with the decision of the mode of phyllotaxis in both untreated and treated bean plant.

Watson (1948) has reported that the effect of the stimulus is not long lasting after treatment of 2,4-D. And, we had also similar results; no effect of methyl ester of 2,4-D was found in the higher parts than the sixth node (see Table 7), and in underground parts, the delaying effect to root system already disappeared at the F_4 -leaved stage of seedling. Therefore, until such time of development, it seems that both methyl ester of 2,4-D adsorbed in plant tissue and the derivatives already metabolized and vanished.

§ 5.—The behaviour of stipules following treatment of methyl 2,4-dichlorophenoxyacetate was phylogenically the most interesting problem of bean plants, since the change of the configuration of stipular and laminal leaves are considered as a clue to analyse the evolutionary step of the mode of shoot-formation.

Maekawa (1949) has pointed out for the first time that the two kinds of leaves, one of which is the cauline origin, derived from the branch through the ancient primitive dichotomy in the main stem, and another of which is the leaf derived from the emergences on the surface of the stem, can be produced in individual plant, i.e. in a species, whereas it was hitherto considered that, in a species, plants could produce only one kind of leaves. The material used

in this study, *Phaseolus vulgaris*, has been known as the plant having two different kinds of leaves, i.e. S- and F-class of leaf, and forming the 'synthetic combination between opposite SS and opposite FF (Maekawa, 1952)', and this mode of formation of vegetative shoot may be regarded as *meta-composed* shoot (Furuya, 1953). Then, the change of the configuration of stipular and laminal leaves was not seen yet in the other investigations with other growth substances such as IAA, 2,4-D, etc. in this laboratory, or in the result of many hitherto published papers. However, it was found here that the configuration in synthesis of two different kinds of leaves could be experimentally changed in a wide variety of pattern after treatment of the chemical.

That is, on the third node of treated bean the situation of stipules and of stipella has brought forth the change to new configurations, i.e. 'typical combined leaves', 'deformed combined leaves', 'simple leaf with stipules', and 'typical simple leaf', whereas, in untreated controls, 'typical associated leaves' are produced on the second node, and 'deformed associated leaves' are successively formed on higher than the second nodes. Therefore, the mode of shoot-formation altered from *meta-composed* shoot to *pseudo-composed* shoot, *pseudo-simple* shoot, *meta-simple* shoot, and *ortho-simple* shoot (see, the definition and examples of these modes in Furuya's paper, 1953). On the other hand, Furuya (1953) offered hypothetically the phylogenical relation between several fundamental modes of shoot-formation which were induced from the results of the organographical analyses in many dicotyledonous plants; here represents partially again, \rightarrow *ortho-composed* shoot \rightarrow *meta-composed* shoot \rightarrow *pseudo-composed* shoot \rightarrow *pseudo-simple* shoot \rightarrow *meta-simple* shoot \rightarrow . This hypothetical relation gave us a suggestion that the chemically induced behaviours of stipules seem to coincide with the route of evolution of heterogenous composed shoot.

But, these interesting behaviours of stipules, which proposed such important problems of phylogeny and of morphogenesis, were observed only on the third node, and any change of the configuration of leaves did not appear on the other nodes. And, it is probably the reason of this phenomena that, in the stem-tip of dormant seed, the meristem from which F_2 -leaf and its stipules will be produced are probably in opportune physiological age to be affected by the stimulus, and that the age of other parts of meristem is too younger or too older to occur such responses. Although it is now impossible to resolve this problem exactly, the research of the mechanism of such a matter seems to be a clue to make clear this phenomenon.

According to these facts, it is thought that the mode of shoot-formation is decided by the activity of unknown metabolic system which is directly affected by so-called growth-regulating substances, and that the progression of the chemical equilibrium of these system gave rise to the evolution of the mode of shoot-formation. Further, it is interesting that, when the dormant seeds of bean were pre-treated with the vapour of methyl 2,4-dichlorophenoxyacetate and were still more treated with the solution of so-called antiauxins, i.e. 2, 3, 5-triiodobenzoic acid, these seedling did not present any change of the configuration of leaves (Furuya, unpublished). And, this result suggests us the existence of native growth-regulating substances and of their antagonists that play important

rôle in morphogenesis and in evolution.

§ 6.—Furuya have also found that, when the seeds of bean were pre-treated with the solution of IAA or 2,4-D, these seedlings could induce the same response as that of methyl ester, i.e. the formation of abnormal structural enlargements in the transitional region between stem and root. Boas (1939) reported that “Durch Eosin erzeugte Brettwurzeln, Wurzelverbänderung bei *Phaseolus*”, and Wilde (1951) presented that, when the seedling of red-kidney beans was treated with soil-application of 2,4-D, they produced the conspicuous shoulders of tissue just back of root-tips. Then these similar phenomena took place in somewhat different parts of plants in consequence of the different time of treatment in their seedlings.

It is, however, found that such structures induced by various chemicals showed same morphological organization in each other, in other word, that these differently originated shoulders are too resemble to be considered that the responses were caused by different mechanism. Thus, it is considered that the physiological age of tissue in the transitional region between hypocotyl and radicle at dormant-seed-stage probably coincides with those back of root-tips at seedling-stage, and that tissues, being in the same age, may occur the response by similar stimulus caused with different chemicals.

In Wilde's experiment, four shoulders of tissue are most often formed on the primary and large secondary root tips, and on small lateral root tips the opposite shoulders are formed, giving the root tips the shape of arrow-heads. While, in our studies, four shoulders of tissue are most frequently produced, and, two opposite shoulders are scarcely formed, but the six or seven shoulders of tissue were often seen.

Summary

In the six grades of application, for 1, 2, 3, 4, 5 and 7 weeks, with the vapour of methyl 2,4-dichlorophenoxyacetate on the resting dry seeds of bean (*Phaseolus vulgaris* L.), a wide range of responses took place in the seedlings, and the following results were obtained.

1. Any influence could not be found in the rate of germination after treatment with the chemical at least within seven weeks.
2. Both in the cotyledonous stage and in the first foliage leaved stage, the significant delays in development were proved in all of the six experimental groups.
3. In the treated plants, the blade of the first foliage leaves became, more or less, thick, pale and veiny, and formed a dark green ruffled band around most of the blade margin. The degree of reduction in size of the first foliage leaves pre-treated for the different periods of exposure was shown by the measurement of the length of mid-rib, the angle between both first lateral nerves, and the length of petiole. The differentiation of mesophyllous cells was fully disturbed, and the normal interveinal tissue was absent or supplanted by “replacement tissue”.
4. In the leaves produced on the higher nodes than the third one, no

anatomical modification was observed, however, the organographical changes occurred in the number of leaflets and in phyllotaxis. The first trifoliate foliage leaf (F_2), if this may be affected by the stimulus, responded always inhibitingly and became to the simple or bifoliate leaf, while the second and third trifoliate foliage leaves (F_3 - and F_4 -leaves) responded promotingly and changed to the multifoliate foliage leaves, which showed many kinds of pattern in shape. But, the fourth and the following successive foliage leaves, F_5 -, F_6 -, ..., did not respond to the stimulus of this chemical. The frequency of occurrence of these responses following treatment for several long hours was discussed.

5. Only in the third node of treated bean, both stipules and stipella represented the various degrees of modification in shape and disposition. This change of configuration of stipular and laminal leaves was considered as a clue to analyse the evolutionary steps of the modes of shoot-formation.

6. In the case of treatment for 4 weeks or more, the conspicuous gross responses, which were the abnormal structural enlargements in the transitional region between stem and root, were observed in the underground parts. The anatomical structure of this protuberances was similar to those of the shoulders of tissue back of the root-tips induced by the soil-treatment of 2,4-D.

7. The after-effect test in the successive generations was done: the effects induced after treatment with this chemical could not appear in the next generation, and even when the treatment was made extending over three generations, no accumulative influence to these formative effects has been observed. In the early stage of germination, the plants pre-treated for four weeks or more lack the abilities of phototropism and of geotropism.

These results show that each organ of treated bean represented different behaviours respectively in accordance with its age of development at the time of treatment, though the every part of seed was exposed similarly in the vapour of the chemical. In general, the period with the chemical was the longer, the more frequently these responses occurred, but beyond a threshold of this period of application this tendency was not seen and the frequency with which these responses occurred was found at constant level.

In closing we wish to express our heartfelt thanks to Dr. F. Maekawa, who furnished us with many instructive advices and criticisms throughout this investigation. Mr. S. Sato granted to publish his valuable microphotographs in this paper, and Mr. M. Takeuchi gave us many facilities with his skillful photography. We also wish to express our deepest gratitude to them.

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Genetical Studies on Rice Plants. XIX

The Third Gene in Apiculus Coloration*

By

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(Received July 20, 1955)

Introduction

One of the most striking characteristics in rice genetics is the anthocyanin coloration in apiculus. The genic interpretation of apiculus coloration published hitherto by several workers, however, is far too complicated and can not be brought together under one general gene scheme. This discordance may be partly due to the difficulty in identification of the hue and shade of colors referred to, however, the main reason seems to lie in the lack of extensive and systematically produced cross combinations.

Formerly the authors (1947, 1951a, 1952a, 1953) produced several crosses from varieties which differed greatly in apiculus coloration, and have since proposed an interpretation that encompasses all data concerned.

According to this interpretation, anthocyanin coloration depends on the complementary effect of gene *C* and *S_p*. The *C* gene is responsible for the formation of chromogen, and the gene *S_p* exerts its modifying effect on *C* and turns the chromogen to anthocyanin. Pigmentation occurs primarily in the apiculus and in the empty glumes as a result of the interaction of said two genes and is located in the cell sap of the epidermal layer of the above sites. The *C* alone causes the sites to brown viz. turn "tawny" at ripening. Five alleles, $C^B > C^{Bp} > C^{Bl} > C^{Br} > c$, have been discovered at the *C* locus and three, $S_p > S_p^d > s_p$, at the *S_p* locus. Therefore, the expression of anthocyanin color characters of the apiculus is determined by the combination of alleles of the *C* and *S_p* loci.

From this point of view, a review of literature by previous workers has been conducted by the authors revealing that monohybrid or dihybrid segregation ratios of apiculus coloration as in the case of 3:1, 9:7, 9:6:1, 9:3:4, 9:3:3:1, or 15:1 (Chao 1928, Hector 1913, Mitra et al. 1928, Parnell et al. 1922, Van der Stock 1908, Yamaguchi 1931 etc.) can be satisfactorily explained by the authors' "*C-S_p* combination" scheme. Further in order to exclude the necessity of introducing of "multiple genes and intensifyer for color shade" theories as advocated by Chao or Yamaguchi the authors have previously reported that they have successfully produced test crosses using same materials as Yamaguchi which come under the aforesaid "*C-S_p* combination."

However, some workers such as Lee (1927) and Chao (1928), notably the

* Contribution from the Plant Breeding Institute, Faculty of Agriculture, Hokkaido University, Japan.

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latter, have reported another ratio of colored to colorless, as in 27:37, indicating that at least three genes are concerned in apiculus coloration. This result however cannot be explained by the $C-S_p$ scheme alone.

The means by which to explain trihybrid segregation under the interpretation of $C-S_p$ is the aim of the present report.

Experimental Results

In order to produce the trihybrid segregation under the authors' genic scheme, several kinds of cross combinations involving foreign varieties have been carried out during the past four years. Among these, the cross combinations in which the colorless foreign variety E-36 was combined with testers A-2 (Apiculus and node are a blackish red purple, under a genic constitution of $C S_p P_n$) and A-26 (Originally colorless. Apiculus color turns tawny at ripening; $C S_p p n$) showed the following segregations.

The F_1 of E-36 \times A-2 shows the same type of coloration as that of A-2, having a colored apiculus and node, while in F_2 , in addition to the parental type of colorations, a new type viz. colorless apiculus with colored node appears. This type, tentatively called "xp-type," is decidedly unique in that the color of the node may develop even in the absence of the apiculus color, which (phenomena) the authors have never observed in cross experiments among Japanese varieties (Figure 1).

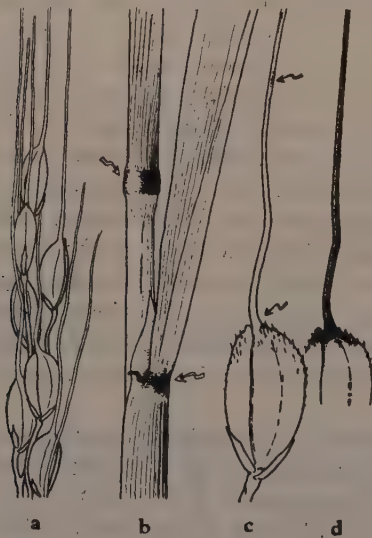


Figure 1. Apiculus, awnes and node color of "xp-type."

a-c: xp-type, based on genic constitution $C S_p a P_n$.

d: Apiculus color based on $C S_p A$.

As shown in enlarged glume (c), apiculus and awnes are dotted with purple stipplings (black arrows, somewhat diagrammatic).

White arrows indicate the location of color by P_n .

Though apiculus is colorless visually, however, slightly colored cells are revealed scattered in microscopical examination; and when the glume is awned a faint hint of purple colors appears in its apex alone, therefore to a cursory view the awn seems also to be colorless. And at ripening the tawny color does not appear in the apiculus or awnes and remains straw white without exception.

The ratio of three coloration types, purple apiculus with purple node, xp-type, and colorless, is approximately 9:3:4, suggesting that it is based on a digenic scheme of inheritance. But in colorless segregants there are some plants which have the same coloration as the xp-type in their apiculus and awnes. This type, tentatively called "x-type," also shows straw color at ripening.

This mode of segregation cannot be

illustrated with the C and S_p combination alone; be that as it may, the fact that the F_2 segregants with colored apiculus are invariably accompanied by colored node suggests the presence of P_n gene in the E-36 variety likewise¹⁾, and the fact that the colors of awnes and apiculus of colorless segregants do not change to tawny in the ripe stage indicates that a certain cause or causes of modifying the effect of the C may exist in genotype of $C S_p$.

In the progenies from E-36 \times A-26, which is the combination between colorless varieties, F_1 shows a colored apiculus and colored node in the same manner as in F_1 from E-36 \times A-2, and F_2 populations are classified under the following five types of coloration with regard to the apiculus and node colors, namely; i) colored apiculus with colored node, ii) colored apiculus with colorless node, iii) xp-type, iv) x-type or colorless apiculus (straw white at ripening), and v) colorless apiculus with tawny ripening color.

As mentioned above, the apiculus coloration of xp-type and x-type may be regarded as colorless, in accordance with the assumption that these two types are colorless, and separating the F_2 populations into colored vs. colorless groups, the actual numbers come to 106:141, which is a close fit to the calculated relation of 27:37 ($p=0.9\sim0.8$). This result gives support to the view that in this cross combination there exists a trigenic inheritance in regards to the apiculus coloration.

When apiculus coloration including the node color is classified, these frequencies are very singular, that is; colored apiculus with colored or colorless node, xp-type, x-type or colorless apiculus and node, and colorless apiculus which turns into tawny, are in numerical relations of 106:30:89:22.

A-2 consists of the genic constitution C , S_p and P_n , and the F_1 from A-2 \times E-36 shows purple apiculus and node. Therefore E-36 must contain the dominant gene S_p and P_n . If so, the determination as to whether E-36 possesses the gene C or not, or the determination as to what genotype of the xp-type remains.

In order to solve these problems, xp-type plants were bred true and the pure breeds were crossed with two testers, $c S_p$ and $C s_p$, with the results of colored apiculus in the F_1 s, indicating that the xp-type possess not only S_p but also C . Therefore, in spite of having identical genotype for C and S_p loci, there exists a monogenic difference between the following two types of apiculus color, colored apiculus with colored node and xp-type in the F_2 from E-36 \times A-2.

This fact indicates that it is necessary to assume that another gene for the expression of the apiculus color in addition to C and S_p exists.

What then is the nature of the action of this new gene, and on which gene, C or S_p , does this new gene (tentative gene symbol " A ") exert its effect? It is most probable that the A has distributing effect on the C , since there is no appearance of tawny coloration in colorless F_2 segregants from E-36 \times A-2. Thus the genic constitution of the xp-type is estimated to be C

1) Anthocyanin color in the stem node develops in the presence of the gene P_n in combination with $C S_p$ (Nagao and Takahashi 1951a).

Table 1. Inheritance of anthocyanin color in apiculus in crossing involving c and a alleles of the C and A loci.

Apiculus color	F ₁	F ₂										Total	χ ²	d.f.	p
		Pheno- type	Colored (Purple)	Colorless in visual, but faintly colored	Colorless		Straw white at ripening	Tawny color at ripening							
Node color		Geno- type	CS _p A*	CS _p α	CS _p a, cS _p A cS _p a, cS _p A cS _p a	Colorless	Colorless	Colorless	Colorless	Colorless	CS _p A	362	1.571	2	0.5~0.3
		Phenotype	Colored	Colorless	Colored	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless				
		Genotype	Pn	pn	Pn	pn	Pn, pn	Pn, pn	Pn, pn	Pn, pn	Pn, pn				
		O	200		62		100								
E-36 × A-2 cS _p Pn a CS _p Pn A	Same as A-2	(3:1) × (3:1)	9	3	4						16	362.00	1.571	2	0.5~0.3
		C	203.62	67.88	90.50										
		O		30	89										
		(27:9:9:19) × (3:1)	27	8(=9 × $\frac{8}{9}$)	20(=9 × $\frac{1}{9}$, 9, 3, 3, 1)										
" × A-26 C _{s_p} Pn A	"		104.20	30.88	77.19						247	247.00	6.830	3	0.1~0.05
		C													

* C denotes C^B or C^{Bp} , as in the other tables.

$S_p Pna$. For the reason in the colorless F_2 segregants of $E-36 \times A-26$ the tawnys outnumber the non-tawnys, it is natural to assume that $E-36$ lacks the dominant gene A and has the genic constitution of $c S_p Pna$.

In accordance with this genic scheme and with consideration to the mode of inheritance of F_1 and F_2 in $E-36 \times A-2$ and $E-36 \times A-26$, the theoretical ratios of F_2 segregations and their deviations from observed number of segregants are as shown in Table 1.

In this table, in regards to the theoretical segregation ratio on node coloration, an 8:1 ratio for colored vs. colorless is shown. As reported hitherto (Nagao and Takahashi 1951b), this is the ratio apriorily calculated from the linkage relationship between the gene S_p and Pn with a recombination value of approximately 18 per cent in the coupling phase.

The observed results are in close accordance with the expectation based on these assumptions.

For further verification of the genic scheme as advocated by the authors, the $H-61$ has been bred from $E-36 \times A-26$ and shows x_p -type coloration and is assumed to have the genic constitution of $C S_p Pna$, was crossed with some tester varieties as described below:

$A-58 (C S_p Pna) \dots$ same as in $A-2$

$A-1 (C s_p pn A) \dots$ same as in $A-26$

$N-44 (c S_p pn A) \dots$ colorless apiculus, straw white at ripening

$A-5 (C S_p pn A) \dots$ pink apiculus with colorless node

The details of the results, being tedious, are abridged here. However as given in Table 2, in all combinations, numerical relations were found between the several coloration types and also between the several classes of behavior which is reasonably close to the expectation.

Furthermore the propriety of these genic interpretation was confirmed by pedigree culture, and in every instance almost all the segregation types in F_3 generation of the above mentioned cross combinations, and no others, have appeared (Table 3).

Table 3. Segregation types of pedigrees and their frequencies in F_3 progenies from the crosses mentioned in Table 2.

a. $H-61 \times A-58$

F ₄ Phenotype	F ₃					Number of plants
	Type of segregation *		Number of pedigrees **			
	IV _p	x _p	C ₁	C ₂	0	
IV _p	1		1	4.5	7	396
"	3	1	2	9.0	7	411
x _p	1	1	1	4.5	4	357
Total			4	18.0	18	1164

b. $H-61 \times A-1$ [illegible]

c. H-61 × N-44

F ₂ Phenotype		F ₃							
		Type of segregation*				Number of pedigrees**			Number of plants
		IV _p	IV	x _p	x, G(t)	C ₁	C ₂	0	
IV _p	1				1	0.3	1	72	
"	3	1			2	0.7			
"	3		1		2	0.7			
"	3			1	2	0.7	1	119	
"	9	3		4	4	1.4	2	204	
"	9		3	4	4	1.4	1	102	
"	9	3	3	1	4	1.4	1	123	
"	27	9	9	19	8	2.8	3	339	
IV	1				1	0.3			
"	3			1	4	1.4	2	128	
"	9			7	4	1.4	2	144	
x _p			1		1	0.3	1	152	
"			3	1	4	1.4	2	169	
"			9	7	4	1.4	2	232	
x, G(t)				1	19	6.5	4	240	
Total					64	22.1	22	2024	
Miscellaneous***							1	22	

d. H-61 × A-5

F ₂ Phenotype	F ₃								
	Type of segregation*					Number of pedigrees**			Number of plants
	IV _p	IV	V	x _p	x, G(t)	C ₁	C ₂	0	
IV _p	1					1	0.3		
"	3	1				2	0.6	2	140
"	3		1			2	0.6		
"	3			1		2	0.6	1	112
"	9	3	4			4	1.3		
"	9	3		3	1	4	1.3		
"	9		3	3	1	4	1.3	3	213
"	27	9	12	9	7	8	2.5	3	303
IV		1				1	0.3	2	145
"		3	1			2	0.6	1	73
"		3			1	2	0.6		
"		9	3		4	4	1.3	2	188
V			1			4	1.3		
"			3		1	8	2.5	1	63
x _p				1		1	0.3		
"				3	1	4	1.3	1	84
"				9	7	4	1.3	2	112
x, G(t)					1	7	2.2	2	140
Total						64	20.2	20	1573
Miscellaneous***								2	195

* IV_p denotes the coloration type as purple apiculus with colored node, IV as purple apiculus with colorless node, V as pink apiculus with colorless node, G(T) as colorless but ripening tawny, and G(t) as colorless and straw white at ripening.

** C₁ indicates the theoretical ratio, C₂ indicates the theoretical numbers, and 0 indicates the observed numbers.

*** These pedigrees contain unexpected segregants or show singular ratios of segregation which may be due to natural crossing or inadequately small plant numbers in determining the mode of segregation.

On the whole, therefore, these results lead the authors to the conclusion that aside from the gene *C* and *S_p* there exists another gene "*A*" for apiculus coloration, and according to this view the expression of the anthocyanin color in apiculus depends on the complementary effect of the gene *C*, which is concerned with the formation of chromogen, the gene *S_p*, which is responsible for the formation of modifier for *C* (chromogen → anthocyanin), and the gene *A*, which is responsible for spreading the chromogen of *C* to the entirety of the apiculus, awnes and the apices of empty glumes. *A* in itself, however, or along with *S_p* does not produce any pigment.

Linkage Relation —

Though to what linkage group the gene *A* belongs is not determined to the authors' satisfaction as yet, a single F_2 progeny resulting from a cross of a H-61 (xp-type coloration, $CS_p Pn A pl$) \times N-45 (purple apiculus and leaf blade, $CS_p pn A Pl$) gives a segregation that undoubtedly shows the occurrence of a linkage between the gene *A* and the gene for leaf blade coloration, *Pl*¹⁾.

As shown in Table 4, the progeny from a selfed F_1 heterozygous for *A* and *Pl*, may be classified into four types of coloration as to apiculus and leaf blade colors, namely purple apiculus with colored leaf blade, purple apiculus with colorless leaf blade, faintly colored apiculus (xp or x) with colored leaf blade, and faintly colored apiculus with colorless leaf blade, totaling 536 plants, in the numerical relations of 415:10:4:107.

Table 4. F_2 of cross between genes for apiculus color (*A*) and leaf blade color (*Pl*), showing linkage relation in coupling.

H-61 ($CS_p Pn pl a$) \times N-45 ($CS_p pn Pl A$)

Apiculus color	Purple			Faintly colored			Total	χ^2	d.f.	p
	A			a						
Leaf blade color	Colored	Colorless		Colored	Colorless					
	Pl	pl		Pl	pl					
Node color	Colored	Colored	Colorless	Colored	Colored	Colorless				
	Pn, pn	Pn	pn	Pn, pn	Pn	pn				
0	415	10		4	78	29	536	6.877	4	0.2~0.1
$(56:1:1:18) \times (3:1)$	224	4		4	54	18	304			
C	394.95	7.05		7.05	95.21	31.74	536.00			

Genotype	<i>A Pl</i>	<i>A pl</i>	<i>a Pl</i>	<i>a pl</i>	Total
0	415	10	4	107	536

R.C.V. = 2.70%

* *Pl* is responsible not only for the coloration in leaf blade but also for the coloration in stem node, ligule, pulvinus, leaf sheath and internode.

These four coloration types should be expected to occur in the relation 301.5:100.5:100.5:33.5 in independent assortment between *A* and *Pl*. The observed deviations from expectations may be caused by *A Pl* linkage in the coupling phase, and the observed result is in close accordance with the

1) The occurrence of the colored leaf blade depends on the action of *Pl* in cooperation with *C* and *S_p* (Nagao and Takahashi 1951c).

expectation based on an approximate 3 percent recombination value between *A* and *Pl*.

Formerly Morinaga et al. (1943) suggested briefly the presence of the linkage relations among the gene for apiculus color (*Ap*), the gene for phenol reaction (*Ph*) and the liguleless (*lg*). As *Ph* and *lg* are inserted into *Pl*-linkage group (Nagao and Takahashi 1952a) it is possible to assume that *Ap* is also linked with *Pl*. As to whether *Ap* is identical with *A* or not, the question is left for the present.

General Considerations

On the basis of above mentioned genic interpretation on trigenic inheritance of apiculus coloration, it is concluded that the reformed *C-S_p-A* scheme proposed as a basic interpretation of the apiculus coloration is substantiated, in so far as it is possible to determine.

It is noteworthy that in connection with the apiculus coloration and in accordance with the *C-S_p-A* scheme, the following two problems which have been debated for a considerable length of time seem to be settled to some extent.

One of these is the explanation for such types of coloration as light colored apiculus with dark colored leaf and blade, and the other is the question as to whether plants with colorless apiculus but with some coloration in other parts exist or not.

If in a plant which has a genic constitution of *C^BS_pA* and shows a dark coloration in several parts¹⁾, *A* is replaced by *a*, and the distribution of colored cells in apiculus becomes scarce, resulting in the shade of the apiculus color showing a phenomenal decrease in comparison with that of the other parts.

And if a plant, which has the genic constitution of *C^{Bp}S_p^dAPl* or *C^{Bp}S_p^dAPn* and shows light coloration in apiculus and other parts such as leaf or node, loses the dominant gene *A*, its apiculus may scarcely show any coloration and in visual observation the apiculus is considered to be colorless in spite of having colored leaf or node.

Summary

1. In this report the authors deal with a new gene which refers to the anthocyanin coloration in apiculus.
2. In addition to the known two series of multiple allelomorphic fundamental genes *C* and *S_p*, there exists another single pair of gene *A*.
3. According to this scheme of the authors, the anthocyanin color on apiculus depends on the complementary effect of the *C*, *S_p* and *A*. *C* is the

1) In combination with *S_p* (exact. *S_pA*), *C^B* gives dark purple color in apiculus, awnes, empty glumes, and stigma, and purple lines on the internodes and the lower part of leaf sheath. With *s_p* (exact. *s_pA*), it makes these parts colorless at the time of flowering and tawny at ripening. (Nagao and Takahashi 1947, 1951a)

basic gene for the production of chromogen, and S_F exerts its modifying effect on C and turns the chromogen to anthocyanin, while A is concerned with spreading the chromogen throughout the entirety of the apiculus and awn.

4. Under logical combinations of complex series of these three pairs of genes, almost every kind of segregation ratio in mono-, di-, tri-hybrid, and the differences in color and shade can be explained reasonably, as far as the authors have examined.

5. Though without certainty, it is suggested that A may be inserted into Pl -linkage group with the recombination value of about 3 percent between A and Pl .

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An Antibiotic isolated from Culture Filtrates of *Gloeosporium Olivarum* grown on Media containing 2, 4-D

By

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1. Introduction

As is well known, a large number of investigations have been made until the present time about the mechanism of the activity of the plant growth-regulators, including 2,4-D, to higher plants. Although there has been also accumulated a relatively enormous amount of literature on their activity to fungi or bacteria, almost nothing is known of the process in them. As far as the writers know, the report of Anker (1) that the respiration of *Saccharomyces cerevisiae* was stimulated by heteroauxin is the only one concerning this problem. In the course of investigations on the effect of 2,4-D to *Gloeosporium Olivarum* Alm. causing the olive anthracnose, the writers found out a tendency that the growth rate of the pathogen on agar media containing 2,4-D decreases with the progress of culture in spite of the absence of such tendency in CuSO_4 or uspulun. Therefore there arose a question that 2,4-D might induce the causal fungus to produce a fungistatic substance responsible for the inhibitory activity of 2,4-D, the suspected substance being increased with the culture age. Recent investigations undergone along this line (3, 4, 6) have confirmed the existence of an antibiotic substance in the culture filtrate of the fungus which was grown in the presence of 2,4-D and it was partly isolated as a yellow oil. The present paper mainly gives an outline of the writers' experimental studies already published in Japanese. The additional results concerning this problem are now in press in English (5).

2. The growth curve of *Gloeosporium Olivarum* on agar media containing 2, 4-D, with special reference on the comparison with uspulun and CuSO_4

Gloeosporium Olivarum Alm. was cultured for 16 days at 25°C on agar media containing 0.01, 0.015, 0.02, 0.06 and 0.32% sodium 2,4-dichlorophenoxy-acetate (2,4-D) respectively in Petri dishes, and the diameter of the colonies was measured each day. Unless otherwise indicated, the basal medium used in this paper is as follows: peptone 20 g, sucrose 50 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.4 g, KH_2PO_4 1 g, 2% FeCl_3 solution trace, agar none or 2%, dist. water 1 L. In the case of the control media not containing 2,4-D, the glass tube presented in

Fig. 1 was also used inoculating at one end of the tube since the measurement beyond 8 days was impossible by the use of Petri dish of the normal size. For comparison with 2,4-D the causal fungus was also cultured on media supplied with uspulun or CuSO_4 , the latter being added on the modified Richards' media as follows: KH_2PO_4 5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2.5 g, $\text{C}_4\text{H}_4\text{O}_6 (\text{NH}_4)_2$ 10.6 g, sucrose 50 g, agar 3%, dist. water 1 L. The resulting curves are shown in Fig. 2, Fig. 3 and Fig. 4.

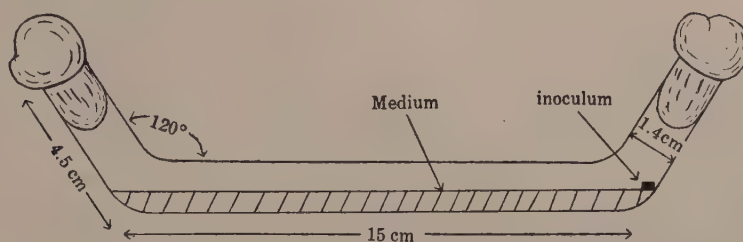


Fig. 1. Growth tube for measuring linear growth.

The control in Fig. 2 alone has been plotted on the basis of a twofold length of colonies in the glass tubes, since the length of colonies on the glass tubes was about the same with the radius of those on Petri dishes at least until the 8th day. In general, the growth of fungi on normal agar media has been recognized to enter into a linear logarithmic phase after a preliminary lag phase. In the present experiments too, a similar tendency was observed not only in control media without these chemicals but also in those supplied with uspulun or CuSO_4 . Moreover the lag phase in uspulun seemed to be prolonged gradually with the increase of concentration.

Experiments with higher concentrations than 0.3% of CuSO_4 were impossible due to its crystallization in media. When the fungi were grown on agar media containing chemicals which exhibit an inhibition by their direct effectiveness against the organisms, the resulting growth curve would probably similar with the case CuSO_4 or uspulun added. As compared with these instances, the growth curve on media supplied with 2,4-D proved to be "log type", consequently the growth rate decreasing with the age of culture. This specific growth phase of 2,4-D did not depend on the change of pH value during

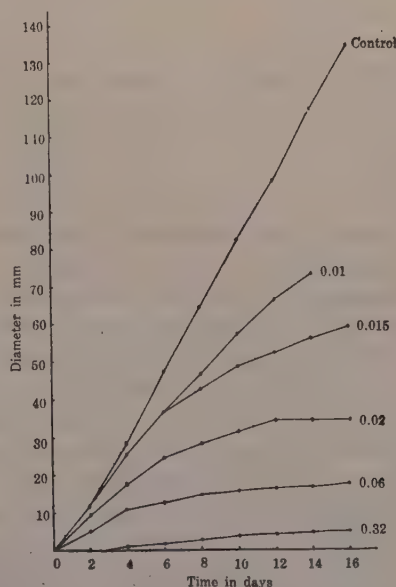


Fig. 2. Growth curves of *Gloeosporium Olivarum* on peptone-salts agar media containing various concentrations (%) of 2,4-D during 16 day's incubation at 25°C.

culture, and also was improbably attributable to the exhaustion of nutrients, because sugars and N source had been remained in a great deal even at the end of culture. Similar relation is also reported by Richards (7) in *Schizophyllum commune*.

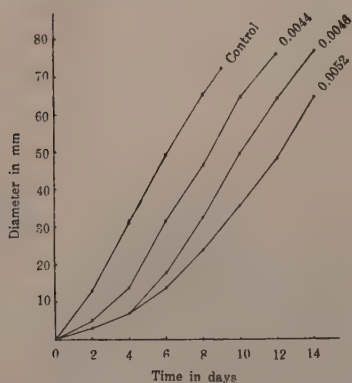


Fig. 3. Growth curves of *Gloeosporium Olivarum* on peptone-salts agar media containing various concentrations (%) of uspulun during 14 day's incubation at 25°C.

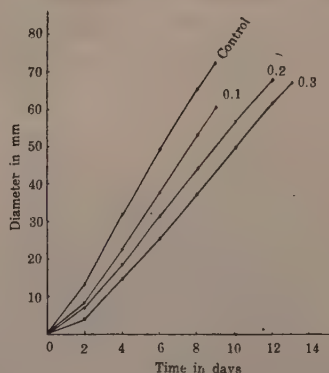


Fig. 4. Growth curves of *Gloeosporium Olivarum* on modified Richards' agar media containing various concentrations (%) of CuSO_4 during 13 day's incubation at 25°C.

3. Effects of filtrates and mycelia of *Gloeosporium Olivarum* obtained from the culture supplied with 2,4-D on its own growth of the pathogen

In view of the specific growth curve of 2,4-D described in the previous section, there occurred a question that a certain staling substance inhibitory against its own growth might be produced when *G. Olivarum* was grown in the presence of 2,4-D, the production of the suspected substance increasing with the progress of culture.

In order to gain a rough information relating this problem, preliminary experiments were undertaken by means of the following methods with interesting results. In the first place, the pathogenic fungus was incubated for various periods on liquid media not containing or containing varying concentrations of 2,4-D (the 1st culture). Then both of mycelia and filtrate obtained by this 1st culture were added to fresh agar media on which the pathogen being cultured to make clear the inhibitory intensity of them (the 2nd culture). The reason that both mycelia and filtrate were together added to media for the 2nd culture was to make the total 2,4-D supplied through the 1st and 2nd culture be identical in each lot, provided that 2,4-D remains unaltered during culture. Prior to the 1st culture, 2,4-D was added to peptone synthetic solution to give the final concentration of 0, 0.01, 0.02 and 0.04%, 50 cc of which solution being poured into each Erlenmeyer's flask of

200 cc quantities, and then being sterilized three days in a Koch steam sterilizer for one hour each day. Withdrawing at random 5 flasks per each concentration of 2, 4-D at 4-day intervals, the harvested mycelia were grounded in a mortar and then resuspended in their own filtrates. To the resulting suspension were added agar and 100 cc of dist. water used for washing of the mycelial residue in a mortar besides the fresh nutrients quite similar with the initial supply in 5 flasks of the 1st culture.

Therefore the concentrations of nutrients newly added prior to the 2nd culture are 250 cc/350 cc of the initial value in the 1st culture, the composition per 100 cc being noted in the foot note of Table I (M). The diameter of colonies in the 2nd culture grown on Petri dishes was measured after incubation of 7 days. The lot containing twofold amounts of the standard medium (M) alone was used as control of the 2nd culture. Furthermore lots devoid of the 1st culture, thus containing neither mycelia nor filtrate of the 1st culture, were also prepared for the purpose of comparison. Hence according to not only the presence or absence of 2, 4 D, its concentration and incubation period in the 1st culture on the one hand but also the concentration of 2, 4-D later added for the 2nd culture on the other hand, the composition of media in the 2nd culture is different each other. The degrees of inhibition in the 2nd culture, expressed in "index", were calculated by dividing diameter of colonies after incubation of 7 days by that on control media, multiplying the quotient by 100.

Experiment 1

21 kinds of media prepared by the above stated method were adjusted to pH 5.4 and 8-10 Petri dishes were used per each lot. The results are shown in Table 1. As already mentioned, the nutrients of the 2nd culture are the sum of the amount remained after the 1st culture and that afterwards newly added for the 2nd culture. Since, however, the residue of nutrients after the 1st culture might not be identical in each lot, the nutritious constituents of the 2nd culture would probably different in each lot. Therefore in order to discuss the data of Table 1 from the standpoint of the inhibition in the 2nd culture, it may be taken for granted that whether such difference of nutrients markedly affected the mycelial growth or not must be confirmed in the first place. Provided that the nutrients supplied had been completely exhausted by the organism during the 1st culture, the initial amount of nutrients of the 2nd culture is only one (M) later added prior to its culture, namely being similar with those of the 8th, 11th, 16th and 21st lot devoid of the 1st culture. On the contrary, if nutrients were not expended at all through the 1st culture, the initial constituents of the 2nd culture media are just double of the case now stated, thus being the same as those of the 1st, 12th and 17th lot. Since, however, it seems unlikely that in this experiment such an extreme case took place, presumably the amount of nutrients at the end of the 1st culture would lie between the two extreme values. Nevertheless in practice, any measurable difference of the growth was not observed between the 1st and 8th, 9th and 11th, 12th and 16th, or 17th and 21st lot respectively as seen in Table 1. Consequently it is evident that in this

Table 1. The diameter of colonies of *Gloeosporium Olivarum* on agar media adjusted to pH 5.4 containing the mixtures of mycelia and filtrates of the causal fungus which was incubated at 25°-27°C for different periods on liquid media supplied with various concentrations of 2,4-D.

Number of lot	Cultural condition				Diameter of colonies in 2nd culture (mm)	Growth index against control
	Concentration of 2,4-D in 1st culture (%)	Culture period of 1st culture (days)	Nutrients newly added for 2nd culture*	Concentration of 2,4-D in 2nd culture (%)		
1			2 M	0.0	60.7	100.0
2	0.0	4	M	0.0	60.0	98.8
3	0.0	4	M	0.014	30.7	50.6
4	0.0	8	M	0.0	63.3	104.3
5	0.0	8	M	0.028	50.3	82.9
6	0.0	12	M	0.0	59.0	97.2
7	0.0	12	M	0.028	46.2	76.1
8			M	0.0	59.9	98.7
9			2 M	0.007	57.7	95.1
10	0.01	12	M	0.007	44.8	73.8
11			M	0.007	64.7	106.6
12			2 M	0.014	58.6	96.5
13	0.02	4	M	0.014	52.2	86.0
14	0.02	8	M	0.014	38.5	63.4
15	0.02	12	M	0.014	34.6	57.0
16			M	0.014	53.6	90.0
17			2 M	0.028	41.4	68.2
18	0.04	4	M	0.028	37.8	62.3
19	0.04	8	M	0.028	20.3	33.4
20	0.04	12	M	0.028	13.5	22.2
21			M	0.028	36.5	60.1

* M: The nutrition is as follows: sucrose 3.75 g, peptone 1.42 g, KH_2PO_4 0.071 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.028 g, FeCl_3 trace, dist. water 100 cc.

2M: Two-fold amount of M.

experiment the difference of nutrients did not give a significant influence upon the growth of the pathogen concerned. Lots belonging to any one of the following 3 groups, viz. the 9th-11th, 12th-16th and 17th-21st are quite identical each other in the total amount of 2,4-D supplied in the course of the 1st and 2nd culture, if 2,4-D was not decomposed at all. Hence the inhibitory activity due to the immediate action of 2,4-D alone should be the same between lots belonging to the same group. Nevertheless, the index of the 10th lot which contains both mycelia and filtrate incubated for 12 days in the presence of 0.01% 2,4-D (its concentration in the 2nd culture becomes 0.007%) are 73.8, indicating the existence of virulence, while the index on the media containing 0.007% 2,4-D alone (the 9th and 11th lot) are 95.1 and 106.6 respectively, proving the absence of notable inhibition. Similarly in the case of 0.014% also, lots supplied with 2,4-D alone did not show marked inhibition, the index being 96.5 (the 12th lot) and 90.0 (the 16th lot) respectively, although the resulting inhibition on those containing filtrate and mycelia

incubated for 4, 8 and 12 days in its presence was comparatively remarkable, the index being 86.0 (the 13th lot), 63.4 (the 14th lot) and 57.0 (the 15th lot) respectively. This tendency is more apparent at the concentration of 0.028%, that is, the index of lots containing the mixtures of filtrate and mycelia were respectively 62.3 (the 18th lot), 33.4 (the 19th lot) and 22.2 (the 20th lot) according to the period of the 1st culture, as against 68.2 (the 17th lot) and 60.1 (the 21st lot) in those 2,4-D alone added. Of the results covering all concentrations of 2,4-D examined, attention will be called to the following facts.

(1) The mixtures of the filtrate and the mycelial mat obtained from culture supplied with 2,4-D exhibit inhibition even though the level of 2,4-D in them is too low for the presence of its own inhibitory activity. Moreover when the level of 2,4-D is relatively high, the resulting inhibition by the mixtures is far more severe than that by 2,4-D alone in them is expected.

(2) The inhibitory potency of mycelia and filtrate concerned on the 2nd culture increases as the growth period of the 1st culture is prolonged.

In regard to the cause of these facts the question would reasonably arise that the pathogen might have a national habit to produce a staling substance injurious upon its own growth even under normal cultural condition. However this assumption was easily neglected by the fact that the filtrate and mycelia cultured on the media not containing 2,4-D developed no inhibition at all in any incubation period of the 1st culture (the 2nd, 4th and 6th lot). Another suggestion that a certain substance accelerating the activity of 2,4-D might be produced in the normal culture would also be improbable since the growth of the 5th or 7th lot is conspicuously better than that of the 21st or 22nd lot.

Experiment 2

In consequence of the results of Experiment 1, it was presumed that in the mixtures of mycelial mat and filtrate there exists a certain substance closely connected with the inhibitory action of 2,4-D. However in the 2nd culture media used for the 1st experiment, there are ones which contain the residue of 2,4-D in the 1st culture. Hence it was impossible by means of this method to know exactly the mere inhibition of such probable substances alone, even if present actually. But an earlier study (2) indicates that 0.028% 2,4-D becomes inactive at the pH value of 8.0. Therefore, in the present experiment the 2nd culture was carried out at pH 8.0 on media containing mycelia and filtrates incubated for different periods in the presence of 0.02 and 0.04% 2,4-D respectively (its concentration in the 2nd culture becomes 0.014 and 0.028%), by this means indirectly removing the activity of 2,4-D itself in the 2nd culture. In regard to other details it was quite similar with the 1st experiment. The results are shown in Table 2. It is here seen that the figures in this table are about the same as those of the corresponding lots in Experiment 1, notwithstanding the fact that the activity of 2,4-D itself can not be expected owing to the pH value of 8.0. As previously reported (2), on the other hand, it has been already ascertained that the growth of this pathogen is almost identical at the pH range between 5.4

Table 2. The diameter of colonies of *Gloeosporium Olivarum* on agar media adjusted to pH 8.0 containing the mixtures of mycelia and filtrates of the causal fungus which was incubated at 25°-27°C for different periods on liquid media supplied with various concentrations of 2, 4-D.

Number of lot	Cultural condition			Diameter of colonies in 2nd culture (mm)	Growth index against control*
	Concentration of 2, 4-D in 1st culture (%)	Culture period of 1st culture (days)	Concentration of 2, 4-D in 2nd culture (%)		
13'	0.02	4	0.014	51.9	85.5
14'	0.02	8	0.014	32.4	53.4
18'	0.04	4	0.028	47.1	77.6
19'	0.04	8	0.028	23.1	38.1
20'	0.04	12	0.028	19.1	31.5

* Control is identical with that of Table 1.

and 8.0. Hence this characteristic that the occurrence of their inhibition is independent of pH value must be said to be a point essentially different from 2, 4-D.

4. Isolation of an antibiotic substance from the culture filtrate of *Gloeosporium Olivarum* grown on media containing 2, 4-D

As described in the previous section, it was confirmed that a certain fungitoxic substance is produced in the culture when *G. Olivarum* is grown on media containing 2, 4-D. Therefore some investigations were performed in order to isolate partially the probable substance. The fungus in question was grown in quiet at 25°C in 200 cc Erlenmeyer flasks, each containing 50 cc of the liquid medium supplied with 0.04% 2, 4-D. Not only the resulting mycelia ground in a mortar after incubation for 12 days but also their hot water, ether or alcohol extracts failed to show any inhibition at all for the growth of the causal fungus even though they were incorporated in agar media. On the contrary, when the filtrate was added to the media, the resulting inhibition was almost identical with the case when mycelia and filtrate were both added together, indicating that the probable substance is located in the filtrate. Since, however, it is probable that 2, 4-D still remains unaltered in the filtrate of the 1st culture, there would arise a reasonable doubt as to whether the resulting inhibition might be due to this residue of 2, 4-D, although some facts neglecting this were described in the previous section. To make this point clearer, 2, 4-D was removed from the filtrate by the solvent fractionation presented in Fig. 5 and the inhibitory activity of each fraction has been examined.

When ether was added to filtrates acidified to pH 2.0 with H₂SO₄, the water soluble fraction (A) in which the residue of nutrients moved did not reveal inhibition. If ether layer was shaken with 4% NaOH aqueous solution,

a yellow oil (D) was obtained after the evaporation of the ether. The yield from the filtrate of 1 L was about 180 mg. The growth of the causal fungus was severely checked on agar media supplied with this substance, the fungus thus being inhibited completely by dilution of 1:5,000 and at 1:10,000 the diameter of colonies being about 40% of that on control media. In addition, it exerted an activity even if diluted to a concentration similar to the original

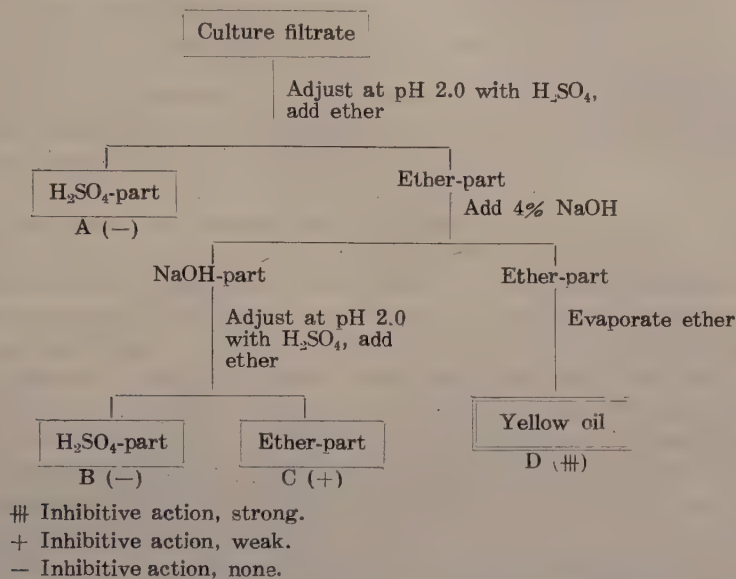


Fig. 5. Process of the partial isolation of the antibiotic.

filtrate. When NaOH soluble fraction was again acidified to pH 2.0 with H_2SO_4 and then shaken with ether, the resulting H_2SO_4 -part (B) did not show a virulence. Although ether soluble fraction (C) slightly retarded the growth of the fungus, the resulting effect would be probably due to 2,4-D since it has been ascertained that 2,4-D transfers to this fraction. The results of this investigation reaffirmed the existence of a fungistatic substance in the culture filtrate obtained in the presence of 2,4-D. Since, on the other hand, this substance in question was not produced even when the media were kept at 25°C for 35 days without inoculation of the causal fungus, it is also fairly clear that the appearance was not due to the simple reaction between 2,4-D and nutrients. Furthermore the substance was not formed even when the filtrate obtained from media without 2,4-D was mixed with 2,4-D. In view of these facts, the substance is considered to be an abnormal metabolic by-product of the fungus owing to 2,4-D. An additional question that its production might be attributable to impurities, because the experiments hitherto described were performed by the use of 2,4-D commercially prepared, was also neglected by the fact that pure 2,4-D crystallized in our laboratory from commercial material also proved to be similarly effective. In addition, this substance was capable of restricting the growth of many other micro-

organisms, too, as shown in the next section. Accordingly, it may be concluded to be an antibiotic substance. The authors' attention is particularly attracted to the fact that *G. Olivarum* is easily induced to form an antibiotic on media supplied with 2,4-D in spite of the nonproduction on those without 2,4-D.

5. The effect of the crude substance upon the growth of microorganisms

The effect of the fungitoxic substance on a number of microorganisms was examined by means of the paper disk method. When the fungus to be tested was grown on agar media until culture plates in Petri dishes were half covered, square filter paper of 1 cm² containing 1.2, 0.6, 0.3, 0.1, 0.06, 0.0 mg of this substance respectively was placed on plate agar near the margin of colonies. After being incubated thereafter at 25°C for 24 hours except in the case of *P. Oryzae* which required 48 hours, the width of a clear zone produced between them was estimated. Because of its insolubility in cold water, the substance concerned was added to paper as a solution of acetone, after its evaporation the paper being provided for use. Since the growth-rates of the pathogen are respectively different from each other, in *C. centrifugum*, *C. edodes* and *S. hydrophilum* the paper was placed at a distance of 3 mm from the ridge of each colony, in *M. Porri* and *C. miyabeanus* at 2 mm and in *P. Oryzae* at 1.5 mm. In the case of phytopathogenic bacteria, on the other hand, the round filter paper of 1 cm² similarly treated was laid on agar plate soon after their suspensions in soy solution were poured on the Bouillon's agar plate, the supernatant fluid being removed. The width of the clear zone was estimated after an incubation period of 48

Table 3. The width in mm of the clear zone produced between the paper disk containing the crude antibiotic and the colonies of the microorganisms.

Microorganism	Micrograms of the crude antibiotic per disk					
	1.2	0.6	0.3	0.1	0.06	0.0
<i>Corticium centrifugum</i>	3.0	2.0	2.0	±	±	—
<i>Cortinellus edodes</i>	3.0	2.0	±	—	—	—
<i>Piricularia Oryzae</i>	0.7	0.5	±	—	—	—
<i>Macrosporium Porri</i>	2.0	1.0	0.5	—	—	—
<i>Cochliobolus miyabeanus</i>	2.0	2.0	1.0	1.0	—	—
<i>Sclerotium hydrophilum</i>	2.5	2.0	1.0	±	—	—
<i>Erwinia carotovorus</i>	4.4	3.0	0.9	±	—	—
<i>Phytomonas destructans</i>	1.6	0.7	±	—	—	—
<i>Erwinia aroideae</i>	7.6	4.8	3.3	±	—	—

± Clear zone, absent; inhibitive action, slight,

— Inhibitive action, none.

hours at 25°C. The results indicate that this substance reveals inhibition against all the organisms tested at the level of more than 3.0 mg as seen in Table 3 and Fig. 6.

The relation of pH to the inhibitory activity of this substance was studied using *G. Olivarum* as a test organism. Filter paper disk supplied with 1.2, 0.6, 0.3, 0.1, and 0.06 mg of this substance respectively was placed on agar media with the distance of 2 cm from the margin of colonies which was previously cultured for five days at 25°C. The width of the resulting clear zone was estimated after successive incubation periods of 24 hours at 25°C. The results showed that the activity of this substance was not inactivated at the pH range of 4.0-11.2.



Fig. 6. Effect of the paper disk containing the crude antibiotic of different amount upon the growth of *Sclerotium hydrophilum*.

1. 1.2 mg 2. 0.6 mg 3. 0.3 mg
4. 0.1 mg 5. 0.03 mg 6. 0.0 mg

6. The production of a clear zone when two colonies of *Gloeosporium Olivarum* were cultured together in the same Petri dish on agar media supplied with 2,4-D

It is a well-known phenomenon that a clear zone is produced at the portion adjacent to the colony of the antagonistic microorganism on account of its antibiotic production when a fungus is cultured together with an antagonistic microorganism on agar media in the same vessel. Since, on the other hand, hitherto described results fairly indicated that *G. Olivarum* produces an antibiotic substance on media containing 2,4-D in spite of nonappearance on media without 2,4-D, the writers assumed that a clear zone would be formed when two colonies of the causal fungus are cultured together on agar media containing 2,4-D in the same vessel, being unformed on media without 2,4-D. To make this clear, two colonies of the present fungus were cultured together on agar media either with or without 0.02% 2,4-D in the same Petri dish, the interval between both inocula being set at 1, 2, 3, 4, 5, 6 and 7 cm respectively (Fig. 7). In each of the two trials, they came into contact independent of the interval between both inocula if they were grown on media unsupplied with 2,4-D. On media with 2,4-D added, on the contrary, they were separated by an inhibition zone with a width of 1.0-3.0 mm even at the end of culture for 30 days. This result is consistent with the conclusion that the present fungus produces an antibiotic substance on media with 2,4-D added. If the inhibition by 2,4-D is merely due to its direct effect upon fungi, it seems to be inexplicable that the clear zone was maintained for so long periods as in the present experiment, even if the unfavourable growth on media with 2,4-D added should be justifiable. The fact that the clear zone was not produced at all when the interval between



Fig. 7. Test for antibiotic production by growing two colonies of *Gloeosporium Olivarum* in association on the same modified Richards' agar plate with or without 0.02% 2,4-D. The interval between both inocula is: 1, 1 cm; 2, 2 cm; 3, 3 cm; 4, 4 cm; 5, 5 cm; 6, 6 cm; 7, 7 cm.

Above, medium without 2,4-D. Age, 13 days.

Below, medium with 2,4-D. Age, 15 days.

inocula was 1 or 2 cm seems to suggest that several days are necessary for the liberation of enough antibiotic to be effective against the pathogen.

Summary

1. When *Gloeosporium Olivarum* Alm., the causal fungus of the olive anthracnose, was cultured for 16 days at 25°C on agar media containing 0.01, 0.015, 0.02, 0.06 and 0.32% 2,4-D respectively, the growth curve of this pathogen on these media was found to be the "log type", indicating that the growth rate of the mycelium progressively decreases with the progress

of culture. On the contrary, the growth phase on media not containing 2,4-D or containing either uspulun or CuSO_4 resulted in the "linear type".

2. If the fungus is cultured on agar media containing culture filtrates obtained in the presence of 2,4-D, the mycelial growth is retarded more severely than when cultured on those to which 2,4-D alone is added. On the contrary, the resulting mycelia do not exhibit an inhibitory activity at all.

3. When the culture filtrate of the causal fungus obtained from liquid media supplied with 2,4-D was acidified to pH 2.5 with H_2SO_4 and shaken with ether and then with 4% NaOH solution, a yellow oil was obtained in partly purified form after the evaporation of the ether. Not only was the present fungus completely inhibited by the dilution of 1:5,000 of this substance, but it also exerted an inhibitory effectiveness upon other phytopathogenic fungi and bacteria tested. Therefore, the substance in question is considered to be an antibiotic substance.

4. When two colonies of the present fungus were cultured together on agar media in the same Petri dish at such intervals as 1, 2, 3, 4, 5, 6 and 7 cm of both inocula, they came into contact at any interval between their inocula if they grow on media unsupplied with 2,4-D. However, on media with 2,4-D added, they were separated from each other, forming an inhibition zone with a width of 1.0-3.0 mm even at the end of culture for 30 days.

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New or Noteworthy Species of Cyperaceae from China

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Half a century has elapsed since Dr. Kükenthal issued an elaborate monograph of Caricoideae in Engler's *Das Pflanzenreich*, soon after the publication of *Les Carex de l'Asie orientale* including a fairly conclusive account of the Eastern and Southern Asiatic Carices given by an excellent French botanist A. Franchet. In this long period of time, a considerable number of taxonomical entities have been added fragmentarily to the Chinese cyperaceous flora which might be called the void of interest as well as that of Africa, by such excellent taxonomists as Kükenthal, Handel-Mazzetti, Nelmès, Ohwi, Kitagawa, Kreczetowicz and Tang. For instance, more than fifty species of Chinese Carices are counted in my list of what published after Kükenthal's *Cyperaceae-Caricoideae*. This number, of course, must allow a margin for growth in number, when we obtain another material from China, we, however, have unfortunately no way of getting more Chinese materials at present, and I therefore prepared an enumeration of the known Chinese species of *Cyperaceae*, laying stress on comparing the Japanese species with the Chinese ones, in co-operation with Japanese and foreign seniors. As I noticed, in this work, that some specimens considered to be in need of description are preserved unidentified in the herbaria of the University of Tokyo, the Kyoto University and the National Science Museum in Tokyo (hereinafter referred to them as TI, KYO and TNS respectively), I describe them in the present paper as a preliminary report of my above mentioned work.

I would like to express my sincere appreciation to Dr. Jisaburo Ohwi for his valuable advices, and to Dr. Hiroshi Hara for careful reading of the manuscript. I am also very grateful to Mr. E. Nelmès of the Royal Botanic Gardens, Kew, and to Mr. H. Keng of the National Taiwan University for sending me some fragments of the type specimens kept in Kew Herbarium and the University Herbarium respectively.

Carex dolichogyne T. Koyama, spec. nova e sectione *Lageniformes* (Ohwi) Nelmès. Visum extradium huius speciei *Carici formosensi* Lévl. et Van't. plus minus propincuum videtur, tamen ab illa spiculis foemineis laxius florentibus, squamis masculis non cucullatis, utriculis longioribus circiter 4.5 mm longis distincte recedit, et abs *Carice ligata* Boott spiculis masculis brevibus 1.5 cm longis, utriculis longioribus satis distat. (Fig. 1)

Herba perennis dense caespitosa, rhizomate brevi lignoso radices fuscas multas emittente. Folia fasciculorum sterilium pluria late linearia usque



Fig. 1. *Carex dolichogyne* T. Koyama.

A. habit; B. a part of culm; C. staminate spikelet; D, E. staminate scale and the apex of anther (E); F. apex of anther; G. pistillate scale; H, I, J. various apices of do.; K. perigynium; L. orifice of do.; M, N, O. transverse sections of basal, middle and apical parts of perigynium; P. achene; Q. apex of achene with style base. (from type)

ad 45 cm longa 4.5-6.5 mm lata rigidula plana unicastata supra marginibusque valde aspera a medio versus apicem gradatim attenuantia apice longe acuminata, vaginis basilaribus pallide fuscis cum nervis multis fuscis fuscopurpureisve mortuis in fibras haud dissolutis et collum rhizomatis dense circumdantibus. Culmi debiles pauci intra folia absconditi et basi foliis fertilibus sparse obsiti 12-22 cm alti 1 mm crassi in sicco laevissimi a medio ad apicem usque spiculosi. Spiculae 4-7 contiguae et superiores 3-4 fastigiatim dispositae; terminalis mascula perbrevis intra spiculas foemineas superiores abdita clavato-linearis vel vere linearis sessilis 10-15 mm longa 1.5 mm crassa flavescent; reliquae foemineae anguste cylindricae linearesve 3-5.5 cm longae maturitate 2.5-4 mm in diametro erectae laxiuscule pluriflorae superiores 2 vel summa tantum sessiles ima cum pedunculo capillari breviter exserto laevi, reliquae brevipedunculatae. Bractee superiores 2-3 squamiformes poculiformesve hyalinae vix vaginantes apice rotundatae praeter summam costa unica ex apice bractee in aristam excurrente, inferiores 2 breviter (5-13 mm) vaginantes foliaceae laminis 5-6 cm longis quam spicula sua aliquantulum brevioribus vel aequilongis. Squamae masculae obovatae non cucullatae flavescentes tenuimembranaceae hyalinomarginatae apice rotundae truncatae margine apicis minute ciliolatae basi cuneatae, costa unica viridi. Filamenta libera adpressa. Squamae foemineae ovales naviculares (2.5-) 2.7-3 mm longae medio minute puberulae 1.5-2 mm latae membranaceae pallide flavescentes inconspicue tenuissime plurinervosae latere pallidiores margine latiuscule scariosae apice rotundatae interdum emarginatae mucronatae cuspidatae basi vix angustatae, costa angusta uninervata distincta. Utriculi erecti lanceolato-lageniformes quam squama sesqui longiores (4.2-) 4.5-4.8 mm longi medio 0.8-1 mm lati membranaceae praeter costas 2 laterales prominentes conspicue multinervi praesertim supra medium adpresse puberuli basi subito cuneato-attenuati crassiusculi cum stipite lineari glabri recti 1/2-2/3 mm longo apice sensim attenuati in rostrum glabrum rectum cylindricum 1/2-3/5 mm longum, ore hyalino bidentulo, dentibus deltoideis. Nux arcte inclusa anguste ovato-rhomboidea cum stipite (2.8-) 3-3.5 mm longa medio fere 1 mm lata vere triquetra in sectione transversali, facie castaneofusca inferne et superne valde concava basi in stipitem teretem rectum fulvum attenuata apice in collum breviter cylindricum fusco-fulvum 0.7-0.8 mm longum apice 2/3-3/4 mm in diametro vertice truncatum sensim producta, stylo brevi basi conico-incrassato, stigmatibus 3 brevibus vix persistentibus.

Hunan: Mt. Yolu-shan (Shûzô Yamazaki, no. 31! — Holotype in TI).

Carex recurvisaccus T. Koyama, spec. nova e sectione *Dispalatae* Ohwi; species sat distincta, *Carici nemostachyae* Steudel ut videtur similis, tamen ab ea utriculis valde recurvis ex toto laevissimis, foliis etiam supra laevibus differt. (Fig. 2)

Rhizoma vix caespitosum crassum fibris fuscis laxè obtectum, radicibus robustis; stolon ignotus sed probabiliter existet. Culmus solitarius centralis erectus 55 cm altus vere triqueter infra medium remote 2-3-foliatus medio 3 mm latus ad angulos acutos scaber. Folia culmi linearia ad 6 mm

lata quam lamina bractee sumae breviora aequilantave basi in vaginas longas 5-12 cm longas antice hyalinas fuscopurpureas culmum sublaxe circumdantes vix attenuata, ligula nulla; folia radicalia multa densiuscule fascicularia culmo sesquiduplo triplove longiora late linearia 7-12 mm lata conduplicatoplane herbacea supra nitidula tricostata subtus glauca unicostata magine scabra plus minus revoluta apice subabrupte attenuata basi longe (ad

15 cm) vaginantia; vaginae basiales dorso pallidae antice hyalinae fuscae fulvaeve haud fibrososolutae. Spiculae usque 6 praeter imam vel interdum inferiores 2 aliquantulum remotas fastigiatim dispositae; terminalis (interdum superiores 2) mascula linearis erecta rufofusca 4-7 cm longa 2-3.5 mm crassa spiculam sequentem excedens dense pluriflora; reliquae foemineae paene erectae cylindricae 7-11 cm longae maturitate 6 mm in diametro laxiuscule pluriflorae inferiores exserte pedunculatae superiores subsessiles, pedunculo gracili sub parte florente sparse scabro. Bractee saltem inferiores 3 foliaceae aequaliter erectae culmum paulo superantes ad 24 cm longae (praeter basem raro brevissime — circ. 5 mm — vaginantem) evaginatae. Squamae masculae oblanceolatae fuscorufescentes apice obtusiusculae margine late hyalinae; antherae 2.8 mm longae. Squamae foemineae linearilanceolatae longe aristatae 2.5-5(-6) mm longae e basi ovatodeltoidea ellipticae latere hyalina rufopurpurea 0.5-0.8 mm lata sursum in aristam valde obcompressam 1/3 mm latam trinervatam superne asperam apice acutiusculam gradatim attenuantes. Utriculi reflexi ex toto valde recurvi squamam parum super-



Fig. 2. *C. recurvisaccus* T. Koyama—A. inflorescence; B. perigynium with its scale; C. lower part of scale; D, E. orifices; F. achene. (from type)

mae masculae oblanceolatae fuscorufescentes apice obtusiusculae margine late hyalinae; antherae 2.8 mm longae. Squamae foemineae linearilanceolatae longe aristatae 2.5-5(-6) mm longae e basi ovatodeltoidea ellipticae latere hyalina rufopurpurea 0.5-0.8 mm lata sursum in aristam valde obcompressam 1/3 mm latam trinervatam superne asperam apice acutiusculam gradatim attenuantes. Utriculi reflexi ex toto valde recurvi squamam parum super-

antes aequantesve raro ea paullo breviores ellipsoidei valde tumidi fere orbiculares in sectione transversali (3.8-) 4.5 5mm longi medio 1.2 1.5mm lati membranacei plurinervi glabri superne brunneovirides in sicco infra medium fuscopurpurei basi subito attenuati brevistipitati apice etiam subito attenuati in rostrum conicocylindricum laeve recurvum, ore hyalino bifido, dentibus patulis apice rotundatis obtusisve. Nux parvula perlaxe inclusa obovata trigona 2.3mm longa basi cuneatoattenuata apice subito contracta mucronulata, stylo longo gracili flexuoso, stigmatibus 3 brevibus excurvis.

Kwantung: Tsengshing District, Mt. Naamkwan-shan (W. T. Tsang, no. 20068!—Holotype in TI).

Carex sendaica Franchet var. **Nakiri** (Ohwi) T. Koyama in Journ. Jap. Bot. 29: 47 (1954).—*C. brunnea* (non Thunb.): Franchet in Nouv. Archiv. du Muséum 3^e sér., 8: 241 (1896) ex pte.; Kükenthal in Engl. Pflanzenr. 4-20: 599 (1909), pro maxima parte, incl. fig. 102, A-E; Nakai, Flor. Korean. 2: 324 (1911)—*C. brunnea* var. **Nakiri** Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. ser. B, 11, no. 5: 467 (1936)—*C. Nakiri* Ohwi in Acta Phytotax. et Geobot. 5: 64 (1936), sine descrip. latin.

Chekiang: Hangchow, Mt. Peikaofeng (H. Migo, TNS. no. 97713!)—Japan, Korea.

This specimen may be the only one of this taxon from China represented in Japanese herbaria. Now, there is no room to doubt about the occurrence of this taxon in China. The Chinese specimens cited by Kükenthal under the name *C. brunnea* in his monograph must contain the present species, for *C. brunnea* in the meaning of almost all authors before Dr. Ohwi is composed of at least two different species, i. e. *C. brunnea* itself and *C. sendaica* as I mentioned in Vol. 29 of the Journal of Japanese Botany.

Carex rubro-brunnea C. B. Clarke in Hook. fil., Flor. Brit. India 6: 710 (1894); Kükenthal in Engler, Pflanzenr. 4-20: 344 (1909).

var. **rubro-brunnea** (C. B. Clarke)—India, Nepal.

var. **brevibracteata** T. Koyama in Bull. Nat. Sci. Mus. n.s. 3: 25 (1956).

Abs *C. rubro-brunnea* var. **taliensi** Kükenth. differt foliis bracteisque flaccidis, bracteis multo brevioribus et inflorescentiam non superantibus, spiculis brevioribus terminali semper mere mascula etc., et a typo speciei foliis bracteisque flaccidioribus, bracteis quam inflorescentia brevioribus, squamis foemineis utriculo subaequantibus praecipue distinguenda.

Kiangsi: Lushan (H. Migo, TNS. no. 97703!).

var. **taliensis** (Franchet) Kükenthal, l. c. 344 (1909)—*C. taliensis* Franchet in Bull. Soc. Philom. Paris 8^e sér., 7: 34 (1895) et in Nouv. Archiv. du Muséum, Paris 3^e sér., 9: 139, t. 2, f. 2 (1897); C. B. Clarke in Journ. Linn. Soc. 36: 313 (1904)—*C. rubro-brunnea* var. **elineolata** Merrill in Lingnan Sci. Journ. 13 (1): 18 (1934), **syn. nov.**—China: Yünnan, Shansi, Hupeh, Kwantung, etc.

The very long persistent stigmas which well characterize the present species are a very rare character in sedges. It is of great interest that this rare character is found in some species of the Section *Graciles* and the two

species, Japanese *C. sadoensis* and the present one, of the Section *Acutae*, there is, however, no phylogenetical relationship between them.

Comparing with var. *taliensis* the isotype of Dr. Merrill's var. *elineolata* (based upon T. M. Tsui no. 74 !), which was separated from var. *taliensis* chiefly by not lineolate perigynia, I noticed that there is no fundamental difference between the two taxa. The resinous spots marked with fine lines on perigynia which are often found in the Section *Acutae* are rather an unstable character.

Carex purpureotincta Ohwi in Acta Phytotax. et Geobot. 2: 159 (1933), in Japan. Journ. Bot. 7: 200 (1934) et in Mem. Coll. Sci. Kyoto Imper. Univers. ser B, 11, no. 5: 451, t. 14 & f. 15 (1936)—Formosa.

var. ***sphaerocarpa*** Ohwi, msc., ex. T. Koyama, var. nova—*C. haematorrhyncha* Ohwi et T. Koy., Bull. Nat. Sci. Mus. n.s. 3: 21, t. 4 (1956)—A typo foliis ad 19 mm latis subtus dense villosis, costis non hispidis, spiculis foemineis laxius florentibus, squamis paullo brevioribus, utriculis maturitate divergentibus reflexisque 3.2–3.5 mm longis haud nitidis e parte inferiori globosa in rostrum lineare rectum 2–2.2 mm longum abrupte rotundatocontractis conspicue distinguitur. (Fig. 3)

Chekiang: Hangchow, Mt. Peikao-feng (H. Migo, TNS. no. 97701 !—Type).

Carex subtumida (Kükenth.) Ohwi in Acta Phytotax. et Geobot. 1: 75 (1932)—*C. ischnostachya* Steud. var. *subtumida* Kükenth. in Fedde, Repert. 27: 109 (1929).

Culm usually solitary from short thick woody decumbent rhizome covered densely with castaneous or purple-brown scales and its fibrous remains, the stolon also woody creeping; leaves few to several to a culm, more rigid than in *C. ischnostachya*, scabrous above and on the margins, longly acuminate at the apex; pistillate spikelets often in fascicle of 2 at the uppermost node; perigynia obliquely patent 3–3.5 mm long, gradually narrowed above to a rather short upright beak terminated by a hyaline 2-toothed orifice; otherwise almost as in *C. ischnostachya*.

Kiangsi: Lushan (H. Migo, TNS. no. 97705 !)—Kiangsu.

This species may possibly be the prototype of our *C. ischnostachya* Steud. known also from China, forming a small distinct Asiatic section *Ischnostachyae* Ohwi with the latter. From the phytogeographical work on Carices, we recognize a group of sedges which are distributed both in Japan and in the Yangtze valley. It is thought that *C. subtumida* and *C. ischnostachya* fall under the category of this distributional pattern, though the two



Fig. 3. A–C. *Carex purpureotincta* var. *sphaerocarpa* Ohwi—A. pistillate scale; B. perigynium; C. orifice. D. perigynium of *C. purpureotincta* Ohwi.

are fairly well differentiated each other. The following species is also given as a good example of this distribution.

Carex aequalta Kükenthal in Engl., Pflanzenr. 4:20: 354, f. 67 (1909); Ohwi in Mem. Coll. I. c. 298 (1936); Ohwi et T. Koyama in Misc. Rep. National Sci. Mus. no. 5: 19, t. 20 (1952).

Kiangsu: Huishan (Huang, s. n. !—TI)—Japan: N. Kyushu, Honshu (Owari, Musashi, & Hitachi).

Carex graminiculmis T. Koyama, spec. nova distincta in sectione *Frigidae* Fries; omnibus speciebus huc usque ex China descriptis spiculis saepissime compositis masculis pluribus utriculis ovalibus valde adpressis ex toto dense hispidis stigmatibus 3 vel 2 ample dissimilis. (Fig. 4)

Herba perennis vix vel laxe caespitans. Rhizoma breve lignosum fibris sordide fuscis parallelis comose vestitum stolones 1-2 validos longos et radices robustas fuscobrunneas emittit. Folia pauca linearia dimidium culmi aequantia ad 35 cm longa 1.5-5 mm lata molliuscula plana unicostata supra nitidula margine recurva minute scabra versus apicem gradatum attenuantia longissime acuminata basi in vaginas longas 5-15 cm longas antice fulvescentes hyalinas culmum arcte circumdantes vix attenuata, ligulis vix productis, ore vaginae basin laminae excedente. Culmus plerumque solitarius erectus 59-70 cm altus rigidulus graciliter cylindricus infra medium 2-2.5 mm latus obsoletissime trigonus usque suborbicularis in sectione transversali vanus multistriatus laevisimus basi foliis paucis et vaginis foliorum mortuorum fulvescentibus fusciscentibusve in fibras parallelas sparse vel vix solutis dense obsitus. Inflorescentia 7-9 cm longa; spiculae 9-21 contiguae vel subfastigiatae paniculatim raro spicatim dispositae; superiores 7-11 masculae rufofulvescentes castaneorubentesve, omnes simplices sessiles vel interdum inferiores 1-2 breviter pedunculatae et inferne floribus foemineis 1-3 instructae, praeter summam tantum oblongoellipsoideam 15-20 mm longam 3-4 mm crassam lineari- vel lanceolato-oblongae 2-23 mm longae 1.5-3 mm crassae apice basique attenuatae acutiusculae densiflorae; reliquae foemineae declinatae pendulaeve oblongae vel obovato-oblongae 8-25 mm longae 3-8 mm in diametro spisse pluriflorae interdum versus basin laxiflorae simplices vel saepe 2-4 apice pedunculi cuiusquam digitatim dispositae, bracteolis squamiformibus ellipticis castaneosanguineis margine late albohyalinis, pedunculis e unica vagina singulis rigidulis laeviusculis longe exsertis ad 3.5 cm longis. Bractea ima foliacea quam inflorescentia paullo brevior vel aequilonga basi breviuscule (1.5-2.5 cm) vaginans, ceterae setaceae breviter vel vix vaginantes. Squamae masculae oblanceolato-oblongae obovato-oblongaeve hyalinae medio sanguineae dorso unicostatae apice marginibusque late albae. Squamae foemineae ovaes vel late rhomboideoellipticae 4-4.5 mm longae medio 2-2.3 mm latae hyalinae nitidae sanguineofuscae vel castaneae margine late albae latere utroque superne ferme breviter sed subdense hispidulae apice subabrupte attenuatae acutiusculae muticae basi cuneatocontractae involucentes, costa angusta tenuiter trinervosa adpresse hispida. Utriculi

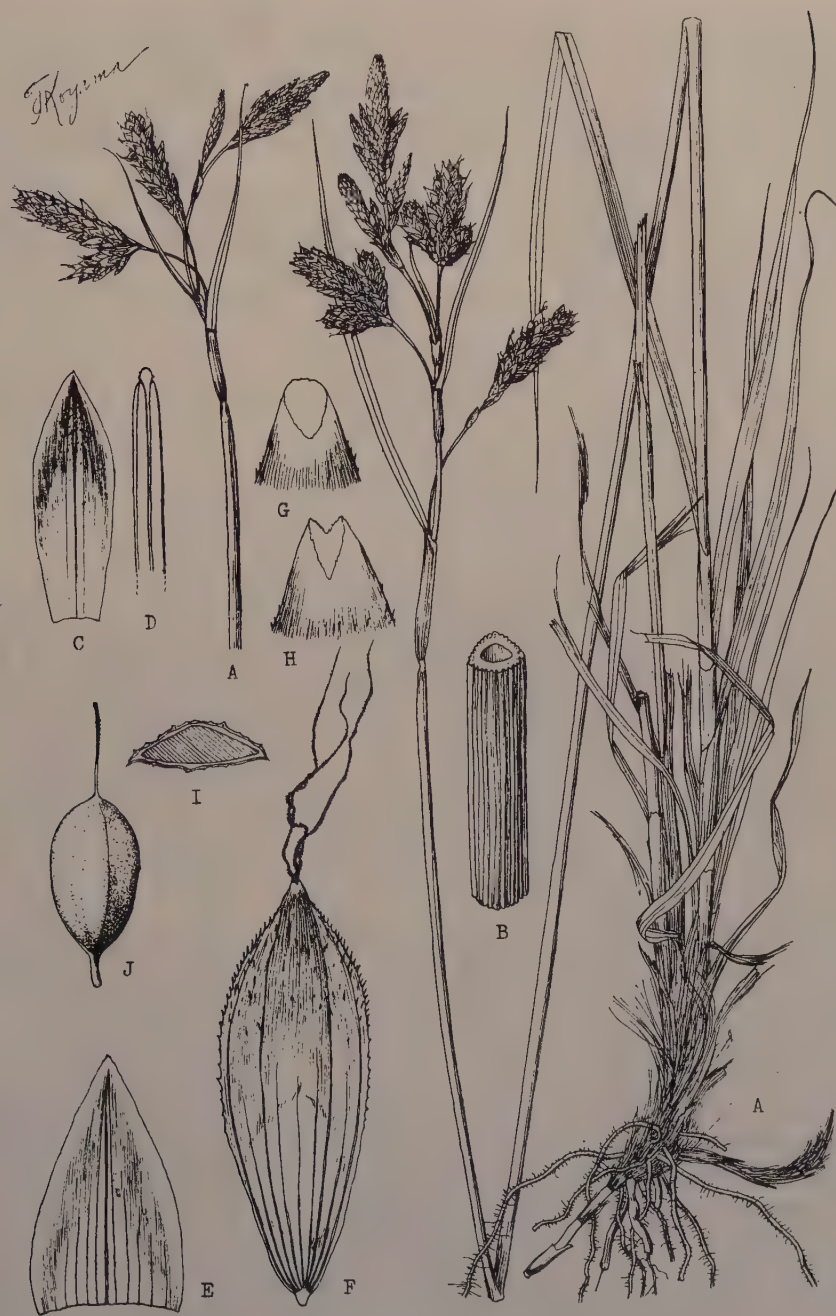


Fig. 4. *Carex graminiculmis* T. Koyama.

AA. habit; B. a part of culm; C. staminate scale; D. apex of anther; E. pistillate scale; F. perigynium; G, H. apices of perigynia showing various orifices; I. transverse section of perigynium; J. achene. (from type)

suberecti valde obcompressi late elliptici ovaesve (4.2-) 5-6 mm longi 2-3 mm lati tenuimembracei 5-7-nervi supra medium fuscopurpurei vel castanei superne et ad nervos marginesque subdense adpresse hispidi basi subabrupte contracti brevissime stipitati apice subabrupte contracti erostrati, ore integro late albohyalino oblique secto. Nux perlaxe inclusa oblonga triquetra raro lenticularis 2.5 mm longa circiter 1 mm lata facie concaviuscula flavens apice subito contracta mucronata basi cuneatoattenuata cum stipite 0.8 mm longo, stylo perlongo ad 3 mm longo flexuoso, stigmatibus 3 raro 2 plus minus persistentibus 2-2.5 mm longis.

Shansi: Mt. Wutai-shan (M. Togashi, 1009!—Holotype in TI).

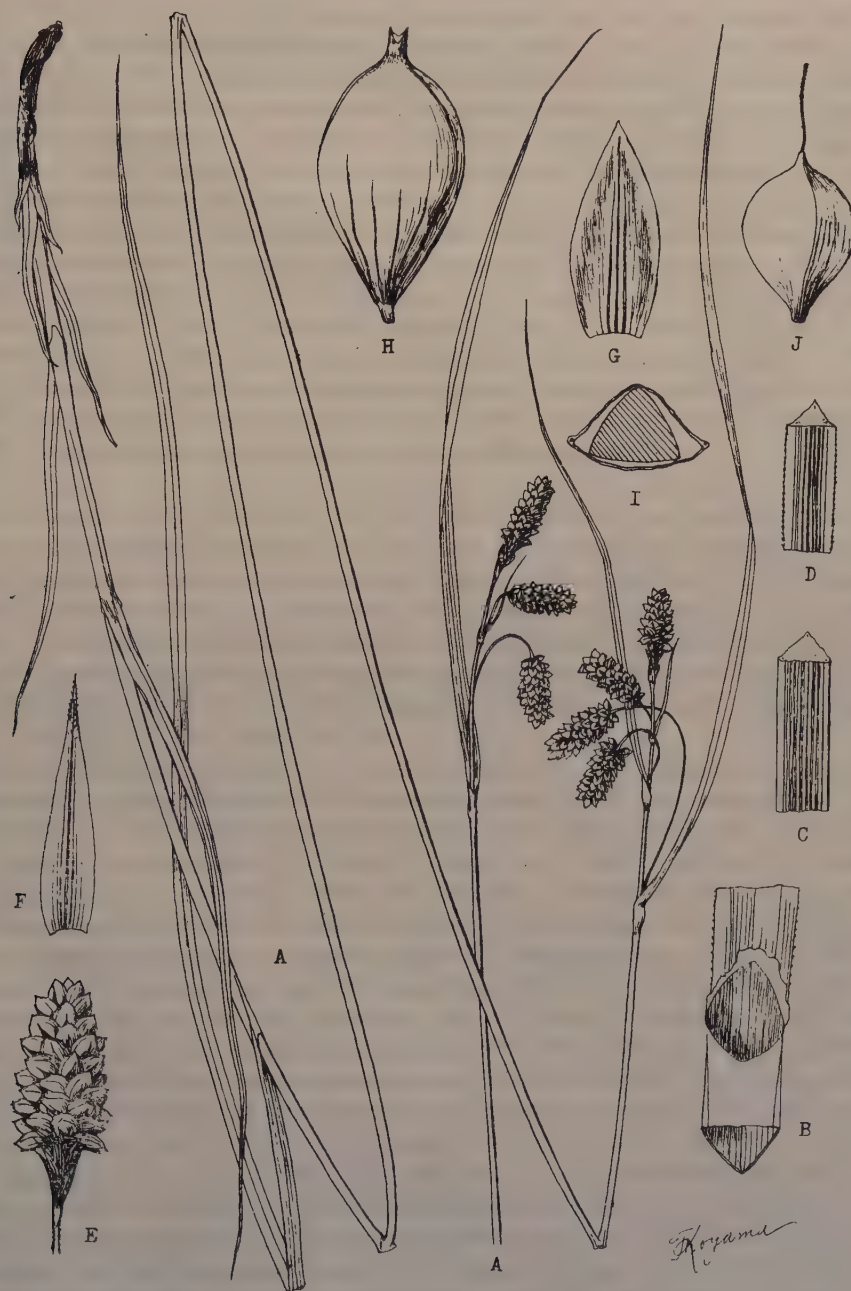
Carex Lehmanni Drejer, Symb. Caricol. 13, t. 2 (1844); Boott, Illustr. Carex pt. 3: 113, t. 361 (1862); Franchet in Nouv. Archiv. l.c. 9: 148 (1897); Kükenth. l.c. 387 (1909); Ohwi l.c. 310 (1936).

Shansi: Mt. Wutai-shan (M. Togashi, 1003!—TI; M. Takahashi, 580!—TI)—Himalaya, Tibet, China: Szechuan, Hupeh and Shensi, n. Korea, Japan.

The distributional area of this species, rather widely extending into Asia from the highland of Himalaya northeastward to Korea and Japan, is a standard alpine type. The above data fill the gap of the area between Shensi and northern Korea (Mt. Setsurei). The Japanese and Korean plants are considerably smaller than the continental ones especially in their vegetative parts. In the plants from Mt. Wutai, culms are up to 6 dm tall and leaves are up to 5 mm broad.

Carex montis-wutaii T. Koyama, spec. nova ex affinitate *Caricis Lehmanni* Drejer a qua ob habitum multo grandiolem, ob spiculas longius pedunculatas majores plus quam 1 cm longas et ob utriculos majores 3 mm longos minus tumidos distincte nervosos brevirostratos satis separatur. Sectio *Atratae* Kunth. (Fig. 5)

Rhizoma ignotum sed probabiliter caespitans. Culmi erecti subvalidi 55-88 cm alti vere triquetri 1.5-2 mm crassi striati acutanguli sursum scabri infra medium laevissimi basi foliis paucis obsiti et breviter decumbentes. Folia linearia dimidium vel $1/3$ culmi aequantia 20-50 cm longa 3-5 mm lata conduplicatoplane mollicula supra subtricostata margine scabra revoluta apice gradatim attenuata longissime acuminata basi in vaginas 6-13 cm longas antice fulvohyalinas ferrugineopunctulatas culmum arcte circumdantes vix attenuata, ligulis ferrugineis breviter ($1/2$ - $2/3$ mm) productis. Vaginae basilares inferiores squamiformes superiores spathaceae aphyllae vel brachyphyllae castaneae vel fuscopurpureae interdum fuscorubentes vix fibroso-solutae. Spiculae 3-4 contiguae vel subfastigiatae; terminalis gynaeandria oblongo-obovoidea 10-15 mm longa parte mascula perbrevis 2-5 mm longa; reliquae mere foemineae oblongae 9-12 mm longae 4-6 mm crassae dense pluriflorae superiores breviter (8-10 mm) inferiores longe (ima ad 5 cm longa) pedunculatae pendulae, pedunculo capillari ad angulos sursum scabro. Bractae inferiores 2 foliaceae inflorescentiam longe excedentes ima 13-18 cm longa, ceterae setaceae, omnes evaginantes. Squamae masculae oblongo-

Fig. 5. *Carex montis-wutaii* T. Koyama.

AA. habit; B. mouth of leafsheath with ligule; C, D. lower (C) and upper (D) parts of culm; E. terminal spikelet; F. staminate scale; G. pistillate scale; H. perigynium; I. transverse section of do; J. achene. (from type)

lanceolatae fuscopurpureae tenuimembranaceae margine late alboscariosae apice acutiusculae dorso cum costa clarius colorata trinervosa saepe plus minus hispida. Squamae foemineae oblongae vel ovato-oblongae 2-2.5 mm longae circiter $2/3$ mm latae hyalinae fuscousanguineae vel fuscopurpureae margine alboscariosae apice subsensim attenuatae cuspidatae, costa concolore vel clarius colorata trinervia. Utriculi divergentes vel patuli squamam multo superantes obovoidei vel ellipsoidei (2.5-) 3-3.3 mm longi medio 1.5-1.8 mm lati membranaceae glabri aurei ex toto dense minute punctulati conspicue 3-7-nervii tumiduli basi subabrupte attenuati cuneati brevissime stipitati apice subito contracti, rostro brevi $1/3$ - $2/5$ mm longo laevi incurvo, ore hyalino bidentulo, dentibus minute deltoideis. Nux subaxe inclusa obovata 1.5-1.8 mm longa 1 mm lata vere trigona facie fuscata concaviuscula basi subito cuneatoattenuata apice subabrupte contracta rotundata vel subtruncata, stylo longiusculo basi aequali, stigmatibus 3 brevibus.

Shansi: Mt. Wutai-shan (M. Takahashi, sin. num.!)—Holotype in TI).

Carex Takenakai Nakai in Journ. Jap. Bot. 19: 318 (1943)—Hopei.

var. **nitens** T. Koyama, var. nova—A typo differt foliis pluribus angustioribus 1.5-2.5 mm latis apice longe acuminatis, spiculis haud fastigiatis longioribus ad 2 cm longis longius capillaripedunculatis declinatis pendulisve, utriculis paullo brevioribus 2.4-2.7 mm longis obovoide oellipsoideis valde inflatis nitentibus apice abruptius brevirostratis, nucibus minoribus.

Shansi: Mt. Wutai-shan (M. Togashi, 1243!; 944!—Holotype in TI).

Carex perakensis C. B. Clarke in Hook. f., Flor. Brit. India 6: 720 (1894) et in Journ. Linn. Soc. 36: 9 (1904); Ridley, Flor. Malay Penins. 5: 184 (1925); Nelves in Kew Bull. 1950: 189 (1950) et in Reinwaldtia 1: 253 (1951); T. Koyama in Le Naturaliste Canad. 82: 195 (1955).—*C. tonkinensis* Franchet in Nouv. Archiv. du Muséum 3^e sér., 8: 251 (1896); Kükenth., in Engl. l. c. 292 (1909)—*C. Dunni* Hayata, Mater. Fl. Formosa: 382 (1911)—*C. Tatewakiana* Ohwi in Acta Phytotax. et Geobot. 2: 299 (1932).—Tonkin, Malaysia, Formosa.

Recently I have seen some fragments of *Carex perakensis* from Clarke's type collection (Malay Penins., Wray!) through the special courtesy of Mr. Nelves of Kew. *Carex tonkinensis* is a rather small-sized plant of *Carex perakensis*. This is a considerably variable species, and moreover as the achenes are very often immature, determination is sometimes difficult.

Carex phyllocephala T. Koyama in Acta Phytotax. et Geobot. 16: 40 (1955); Ohwi et T. Koyama in Bull. Nat. Sci. Mus. n. s. 3: 24 (1956).

Chekiang: Mt. Hsi-tienmu-shan (H. Migo!, TNS).—Japan (cult.).

This record is very interesting from the phytogeographical point of view. Last year, I described this new species from a plant cultivated in Kyushu by Mr. Mankichi Harada who transplanted various domestic and foreign plants to his garden in the Province of Hizen, Kyushu. Thus the locality of this pretty species has not been known up to the present, though I expected that this plant must be a native of Central or Southeastern China, for

example Chekiang or Fukien, from the distribution of other members of the Section *Scleriiculmes* Nelmes in which I placed this species. *C. phyllocephala* thoroughly exhibits a peculiar character of sedges upon which the Section *Scleriiculmes* was based. Namely, the lower 3/4-4/5 part of the smooth stout hard yellowish culm is surrounded by dark reddish-purplish bladeless sheaths and the leaves of the normal kind are very densely aggregated on the apical part of the culm only. Taxonomy and geography of the Section *Scleriiculmes* were given in my previous revision of the section published in the Vol. 16 of *Acta Phytotaxonomica & Geobotanica*. Some members of this section are of value of cultivation for their strange look.

Carex nachiana Ohwi in *Acta Phytotax. et Geobot.* 2: 103 (1933) et in *Mem. Coll. Sci. Kyoto Imper. Univers. ser. B.*, 11, no. 5: 470 (1936).

Kiangsu: Suchou (S. Oka, no. 228!—TI)—Japan: Kyushu (Is. Yakushima, Prov. Chikuzen), Honshu (Prov. Kii, Prov. Mikawa, Is. Iki).

This distinct species of the section *Graciles* has hitherto been known only from the western part of Japan. In Japan, *C. nachiana* occurs discontinuously in Atsumi Peninsula, the west coast of Kii Peninsula and the Province of Chikuzen in the northern part of Kyushu. This distributional pattern is thought to be similar to that of *Myrsine stolonifera* (Koidz.) Walker which is recorded in Kii Peninsula, the Province of Nagato, Yakushima Is., Formosa and the Central and Southern China. *Carex nachiana* must be a so-called relic species of the tertiary flora which was more widely extended in the Eastern Asia, Central China to Japan, in the Tertiary. The Section *Graciles* is one of the most difficult group of sedges and is very well differentiated in the southwestern mountainous district of the China proper. The strikingly large ovate-oval perigynia are the chief distinguishing character of this species.

The next one, *C. Henryi*, is also a member of *Graciles*. It is distinct from its nearest species *C. longicruris* by its smaller perigynia with the less scabrous beak and its looser panicle. I give here its description with a figure, for there is no precise description of this species except Franchet's original one.

Carex Henryi C. B. Clarke ex Franchet in *Nouv. Archiv. du Muséum 3^e sér.*, 8: 243 (1896)—*C. longicruris* Nees var. *Henryi* (C. B. Clarke ex Franch.) Kükenth. in *Engl., Pflanzenr.* 4-20: 603 (1909).

Tufted. Rhizome woody abbreviated. Culms erect and curved above 8-11 cm high 1.5 mm thick below hard obsoletely sulcate smooth sharply 3-angled distantly 1-2-leaved below the middle clothed in the basal 12-20 cm with leaf-sheaths and its subtire to fibrous remains. Leaves basal and subbasal and always 1-2 on the culm, mostly much shorter than the culm linear 2.5-4 mm broad stiffish flattish 3-ribbed more or less scabrous upper subabruptly attenuate to a somewhat obtuse tip, the margins scabrous usually more or less revolute, the sheath long tightly surrounding the culm membranous and brownish in front, ligule very short. Basal sheaths bladeless purplish to dark brown splitting into reticulate fibers in front. Inflorescence suberect and

cernuous above, a rather slender compound interrupted panicle occupying the upper 35-43 cm of the culm, consisting of up to 35 secondary panicles at 5-7 nodes; secondary panicles 3-4-nate, middle ones 5-7-nate, cernuous oblong-obovoid rather lax; branches and branchlets capillary slightly compressed scabrid. Bracts the lower 1-2 foliaceous much exceeding its



Fig. 6. *Carex Henryi* C. B. Clarke—
A. a partial panicle; B. pistillate
scale; C. perigynium; D. achene.
(Henry, 4266)

partial panicle but much shorter than the inflorescence 10-15 cm long longly (3-4 cm) sheathing at base, remainder setaceous rather longly sheathing at base; bractlets a hyaline slender bladeless sheath about 5 mm long. Spikelets androgynous cylindrical 8-20 mm long 2-3 mm thick subax-flowered, the staminate part much shorter than the pistillate part 3-4 mm long 3- to several-flowered. Pistillate scales elliptical or ovate-oblong 2.3-2.7 mm long cymbiform flattish above brownish often with somewhat ferrugineous spots thin translucent obtusish to acutish at the apex with narrowly white scarios margins and 1-nerved midrib. Perigynia erect narrowly elliptical or ovate-oblong 2.7-3 mm long 0.8-1 mm broad biconvex or planoconvex reddish-brown when mature thin-textured glabrous except on the margins where subdensely hispid-scabrid usually in the upper 3/4, 5-8-nerved tapering below to a short cuneate pseudostipe about 1/4 mm long, gradually attenuate above to a long upright slender

beak 0.8-1 mm long subdensely hispid on the both margins terminated by a hyaline oblique orifice. Achene tightly inclosed lenticular elliptical to obovate-elliptical about 1.5 mm long 0.9 mm broad yellowish

rounded to apex subabruptly narrowed at base; style long slender; stigmas 2 almost as long as the perigynium persistent. (Fig. 6)

China: Chekiang, Hupeh (!), Szechuan (!), Kweichow, Yunnan (!).

Carex Jisaburo-Ohwiana T. Koyama, spec. nova—*C. Duthiei* (non C. B. Clarke): Ohwi in Japan. Journ. Bot. 7: 194 (1934) et in Mem. Coll. Sci. Kyoto Imper. Univers. ser. B., 11, no. 5: 316 (1936); Akiyama, Caric. Far East. Reg. As. 112, t. 94 (1955).

Perennis laxae caespitosa. Rhizoma abbreviatum plus minus ascendens fibris fuscis laxae obtectum. Folia linearia pauca culmo breviora 4-5 mm lata plana vel planiuscula laevia mollicula apice subabrupte acuta basi vaginantia. Vaginae basillares aphyllae subaphyllae inferiores fuscopur-

pureae squamiformes plus minus nitidae, superiores purpureorubrescentes, demum in fibris atrofusces sparse solutae. Culmi robustiusculi erecti sed apice curvuli 25-50 cm alti vere triquetri praeter partem apicalem scabridam laeves raro medio unifoliati. Spiculae (3-) 4-6 praeter imam tantum cum pedunculo longo tenui remotiusculam pendentem approximatae subfastigiatæ; terminalis gynacandra oblonga parte mascula perbrevis; reliquae foemineae oblongocylindricae 12-25 mm longae circiter 5 mm in diametro dense pluriflorae superiores subsessiles inferiores breviter exserte pedunculatae omnes cernuae. Bractee ima subfoliacea inflorescentiam superans, ceterae setaceae usque squamiformes, omnes evaginantes. Squamae foemineae ellipticae vel ovato-oblongae (3.2-) 3.5-3.8 mm longae medio 1.1-1.3 mm latae naviculares atro-vel fusco-purpureae opacae dense puncticulatae ad margines angustissime clarius concolores basi abrupte angustatae plus minus involucentes versus apicem subsensim attenuatae et apicem acutam interdum breviaristellatam formantes, costa angusta uninervata. Utriculi paullo breviores latioresque (raro aequilati) patentes obovati vel elliptico-obovati 3-3.5 (-3.8) mm longi 1.7-1.9 mm lati adpressi trigoni subinflati membranacei infra medium luteovirides sursum fuscopurpurei ex toto dense punctulati utrinque tenuiter sed distincte 5-9-nervi basi subsensim cuneatoattenuati subestipitati apice abrupte rotundatocontracti, rostro recto brevissimo, ore integro vertice vel oblique secto. Nux laxa inclusa elliptica obovatoellipticae 1.9-2.1 mm longa circiter 1 mm lata vere triquetra facie concaviuscula stramineofusca basi cuneata apice subabrupte contracta, stylo recto longiusculo, stigmatibus 3 breviusculis papulosis.

Abs *Carex Duthiei* C. B. Clarke utriculis multo majoribus obovatis distincte plurinerviatis apice abrupte rotundatocontractis brevissime rostratis sat distincta et abs *Carex Schneideri* Nelmes (e descriptione) differt habitu minore, utriculis obovatis distincte nervatis. (Fig. 7)

Formosa: Mt. Niitaka (J. Ohwi, 3671!—Holotype in KYO; J. Ohwi, 3707!—KYO); ibid. (M. Tagawa, 444!—KYO). Endemic in Formosa.

Mr. Nelmes was kind enough to send me two spikelets of *Carex Duthiei* from Clarke's type specimen (India, Duthie no. 4499!). *C. Duthiei* is apparently distinguishable from the Formosan plant chiefly by far smaller elliptic perigynia almost nerveless and less yellowish. It is my great pleasure to name this fine species in honour of Dr. Ohwi, my early mentor.

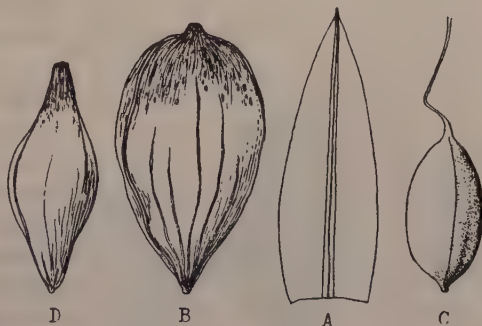


Fig. 7. A-C: *Carex Jisaburo-Ohwiana* T. Koyama—A. pistillate scale; B. perigynium; C. achene. (type). D. perigynium of *C. Duthiei* C. B. Clarke (type).

Radicales (Kükenthal) Nelmes. (Fig. 8)

Densely tufted. Rhizome shortly decumbent oblique woody covered with brown fibers densely; roots fibrous but stout. Leaves crowded numerous basal slenderly linear longer to much longer than the culms up to 30 cm long 1-1.7 (-2) mm wide flattish or plicate sometimes involute-margined rather

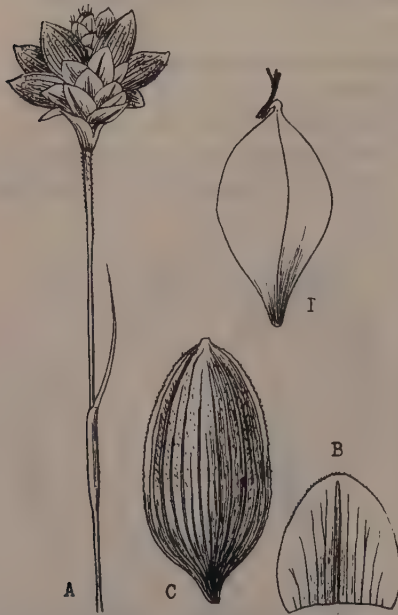


Fig. 8. *Carex Tsoi* Merrill et Chun—
A. inflorescence; B. pistillate scale;
C. perigynium; D. achene. (Fung,
20080)

soft glaucous-green several-nerved on the upper surface and strongly 1-ribbed on the lower surface nearly smooth but scaberulous towards the longly attenuated apex; sheaths rather short 0.8-1.5 cm long brownish splitting into parallel brown fibers later; ligule very short brownish-hyaline. Culms arising from the axil of leaf capillary 4-17 cm tall less than 1/10 mm thick curved and bented down later obtusely trigonous with 1-furrowed surfaces smooth below the middle finely spinose-scabrid above clothed at base with 1 to 3 scaly scarious sheaths. Spike usually solitary terminal androgynous globose to depressed-globose light stramineous-greenish 3-4 mm as long as broad in maturity, subtended below its body by 8 to 12 empty scales (or reduced bracts!); staminate part inconspicuous oblong 2-2.5 mm long about 0.8 mm thick dense-flowered; pistillate part subdensely 4-9-flowered. Bracts 1-3, the uppermost one, just below the spike, glumaceous thin-textured about half as long as the spike amplexicaul at base obtusish at tip; the

others, if present, at nodes 25-35 mm or more distant from one another setaceous shortly sheathing (3-6 mm) below, all empty. Staminate scales tightly imbricated broadly semicircular cucullate rounded at apex finely ciliate on the upper margin. Pistillate scales elliptical to oval herbaceous cinereous 1-1.2 mm long 2/3 mm broad cymbiform rounded to very obtuse at apex obsoletely many-nerved on the both sides with a thickish 1-nerved midrib. Perigynia 3 times longer than the scale patent to spreading ovoid-ellipsoid or ellipsoid 1.9-2.2 mm long 0.8-1 mm broad cinereous-green membranous wholly puberulent on the dorsal two faces and nearly glabrous on the ventral face strongly many-nerved besides the two prominent ribs on the dorsal faces, the margin narrow sparsely hispid from near the base upwards, base subabruptly cuneate-attenuate not stipitate, apex abruptly contracted conical beakless, orifice subentire. Achene tightly inclosed elliptical 3-angled 1.7 mm long 0.8 mm broad with concave yellowish-brown faces, style short abruptly bented at the base scarcely thickened, stigmas 3 brown short.

Hainan: Ling Shui District, Chin Shan (H. Fung, no. 20080 !—TI, KYO !).

This very interesting sedge, known only from Hainan, was included by Merrill and Chun in the section *Microcephalae* Th. Holm, a group of so-called *Primocarex*, by its frequently unispicate inflorescence. But the trigonous, pubescent, narrowly winged perigynia disposed in the distantly spaced androgynous spikelets subtended by several empty scales or much reduced bracts (!) and the some empty bracts on the culm prove this species to be better regarded as a member of *Radicales* which is a small group of Asiatic sedges, ranked by Mr. Nelmès in 1951 by a sectional status. The known members of this section are *C. cylindrostachys* Franchet (China), *C. Delavayi* Franchet (China), *C. hispidangula* T. Koyama (Indo-China), *C. pterocaulos* Nelmès (Burma), *C. radicalis* Boott (Himalaya), *C. speciosa* Kunth (SE. Asia) and *C. stenura* Nelmès (Borneo). In some of these sedges, spikelets reduce to 1 to 2, and the empty bracts found in *C. pterocaulos* and *C. hispidangula*—vestigial cladophylls frequently remain at its axil in the latter—show well the process of the reduction of spikelets.

According to Mr. Nelmès, *Radicales* may be a group of sedges descended from some Indocaricoid ancestor and is not closely connected to *Digitatae*, to which subsection this group was first attributed by Dr. Kükenthal. It is of great interest that the unispicate plants of *Radicales*, for example *C. Delavayi* or *C. Tsoi*, show the Primocaricoid shape. Taxonomically speaking, many species often treated as *Primocarex* are frequently more closely related to some of *Eucarex* or *Vignea* than to any other members of *Primocarex*. For instance, *C. pyrenaica* and *C. grallatoria* are near to *Fuliginosae* and *Digitatae* respectively and *C. gynocrates* bears a close resemblance to *Stellulatae*. Thus, it may be said that the majority of *Primocarices* is derived from some *Eucarex*, *Vignea* or the other genera of the subfamily *Caricoideae*, namely *Primocarex* is not a natural group and is containing a considerable number of "ultimate species" (or unispicate type) of the other groups of sedges.

Carex gentilis Franchet in Bull. Soc. Philom. de Paris 8^e sér., 7: 84 (1859).

var. **Nakaharai** (Hayata) T. Koyama, st. et comb. nov.—*C. Nakaharai* Hayata, Mater. Flor. Formosa: 387 (1911) et Icon. Pl. Formos. 6: 127, f. 40: a-d (1916)—*C. gentilis* (non Franchet): Ohwi in Japan. Journ. Bot. 7: 195 (1934) et in Mem. Coll. Sci. Kyoto Imper. Univers. ser. B., 11, no. 5: 465 (1936); Akiyama, Caric. Far East. Reg. As. 101, t. 78 (1955).

Formosa: rather frequent on mountains. Endemic in Formosa.

C. Nakaharai which has hitherto been considered to be identical with *C. gentilis* occurring in Kiang-si and Yunnan, is fairly well distinguished from the plants from Yunnan chiefly in having slightly larger broadly ovate perigynia about 2.5 mm long with the shorter beak at the apex, broader achenes and slightly taller culms scabrous above. The members of the section *Graciles* are classified by very delicate characters, so the determination is almost impossible without examining many specimens. The sedges treated as *C. brunnea* in Malaysia must be a different species at least from *C. brunnea* itself.

Carex sublateralis T. Koyama, spec. nova e vicinia *Caricis tatsutakensis* Hayata a qua squamis foemineis deltoideis, utriculis brevioribus minus quam 5 mm longis supra medium hirtellis non nitidis obtusius trigonis, nuce late obovato, squamis masculis non connatis satis distinguitur. Sectio *Rhomboidales* Kükenthal. (Fig. 9)

Perennis laxae caespitans. Rhizoma lignosum breviuscule decumbens fibris fuscopurpureis obtectum radices fibrosas multas emittit. Folia normalia omnia fasciculum sterilem formantia linearia 13-25 cm longa 2.5-3.5 mm lata conduplicatoplane primo mollicula sed dein suprigida biennia ad apicem subabrupte attenuata acuta basi in vaginas longas antice hyalinas fuscopureas vix angustata, ligulis subauriculatis hyalinis 1-1.5 mm longis fuscis fuscorubentibusve margine clarius corolatis. Vaginae basilares usque 4, inferiores spathaceae superiores vaginiformes summa tantum breviter (ad 1.5 cm) laminata ceterae aphyllae fusco- vel brunneo-purpureae demum in fibras parallelas dissolutae et collum rhizomatis circumdantes. Culmi e unico

fasciculo circiter 4 inaequales laterales debiles graciles humiles intra folia absconditi erecti ascendentesve 3-5.5 cm alti 0.3-0.5 mm crassi striati laeviusculi fere ex toto vaginis obsiti. Vaginae culmorum 3-4 inferiores vaginiformes superiores subcylindricae 0.5-4.5 cm longae praeter summam breviter laminatam aphyllae fuscopurpureae culmum sublaxe vestientes. Spiculae 3 omnes apice culmi subfastigiatae; terminalis mascula parvula et inconspicua linearilobata 3.5-5 mm longa 0.8 mm crassa pauciflora quam spiculae foemineae brevior subsessilis; ceterae foemineae obovoideae oblongaeve 5-8 mm longae 3-5 mm in diametro sublaxe (2-) 3-florae subsessiles vel interdum cum pedunculo brevi sursum sparse hispidulo erectae vel erectopatentes. Bracteae plerumque 2 patentes inflorescentiam paullo superantes vel subaequantes breves 0.9-2 cm longae 1.3-2 mm latae basi subevaginantes raro ima tantum brevissime (circiter 1.5 mm) vaginans. Squamae masculae oblanceolato-oblongae tenuimembraceae fusciscentes apice obtusiusculae dorso uninervatae. Antherae lineares fere 3 mm longae, connectivo deltoideo. Squamae foemineae parvae late ovatae

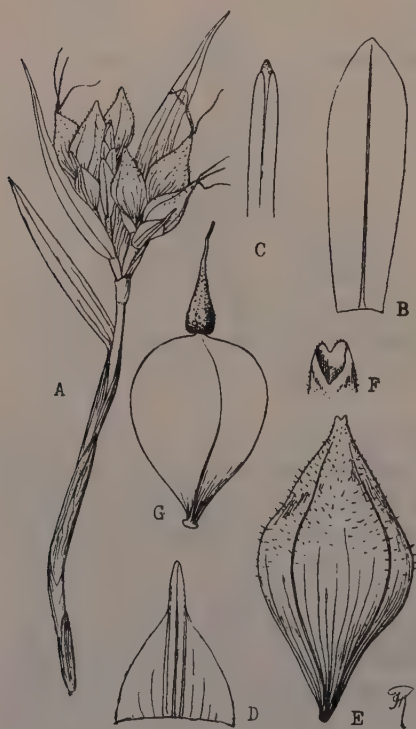


Fig. 9. *Carex sublateralis* T. Koyama:—

A. culm with inflorescence and sheaths; B. staminate scale; C. upper part of anther; D. pistillate scale; E. perigynium; F. orifice of do.; G. achene. (All from type)

vel deltoideae 2-2.5 mm longae 1.7-2 mm latae latere utrinque membranaceae obsolete paucinervulosae a basi ad apicem subabrupte angustatae apice e carina lata laete viridi trinervata in aristam latam breviusculam laevem obtusam excurrentes. Utriculi suberecti majores squamam subtriplo superantes deltoideo-ovales vel late rhomboidei 4.5-4.8 mm longi 2.3-2.5 mm lati subcompressi trigoni membranacei antice distincte bicostati infra medium glabri et tenuiter multinervioli basi cuneatoattenuati sessiles supra medium subdense fuscohirtelli et praeter costas 2 nervii ad apicem subito contracti deltoidei, rostro brevi recto, ore hyalino obtuse bidentulo. Nux subaxe inclusa late obovata vel obdeltoidea 2.8-3 mm longa 2 mm lata facie concaviuscula clarius glauca puncticulata basi abrupte cuneatoattenuata subsessilis apice depresso rotunda, stylo subbrevis flexuoso basi spongiosoincrassato anguste pyramidato demum deciduo, stigmatibus 3 longiusculis.

Kiangsu: Mt. Shangfang-shan, nr. Suchow (K. Kimura, sin. num. 1!—Holotype in KYO).

This strange species is more or less related to *Carex tatsutakensis* which was first described from Formosa by Dr. Hayata and recently reported also from Tonkin by the present writer. *Carex Loheri* C. B. Clarke (Philippines), *Carex lateralis* Kükenthal (India and Malaysia), *Carex macrandrolepis* Lévêillé (Japan, southern Korea and Formosa), *Carex tatsutakensis* Hayata (Formosa and Tonkin) and the present one seem to form a natural small group of sedges distributed in the southeastern part of Asia by their lateral slender culm surrounded below by several bladeless sheaths and often far shorter than the sterile leaves, their perigynia at least smooth below and having somewhat waxy gloss, and their achenes broadly obovoid crowned by narrowly pyramidal stylebase spongy and deciduous later. These have been included in the Section *Rhomboidales* Kükenthal, Dr. Ohwi, however, placed three of these sedges growing in Japan in the Section *Digitatae* Fries in his monographical work. These sedges, in fact, show the intermediate habit between *Digitatae* and *Rhomboidales*; namely except their long-bladed bracts and distinctly beaked, often glabrous, rhomboid perigynia, their habits are rather near to those in *Digitatae*. The Section *Rhomboidales* Kükenthal in its original sense is considerably a heterogeneous section, yet none of us succeeded to divide it into two or more groups. So, I reluctantly retained these species in the Section *Rhomboidales* Kükenthal.

Cyperus compactus Retzius, Observ. 4: 10 (1789); Kükenthal in Engl., Pflanzenr. Heft 101: 423 (1936); Ohwi in Mem. Coll. Sci. Kyoto Imp. Univ. ser. B, 18, no. 1: 159 (1943)—*Mariscus Tamakugu* Masamune et Syozi in Acta Phytotax. et Geobot. 14: 89 (1951), **syn. nov.**

Through the courtesy of Mr. Keng, I was able to examine the holotype of *Mariscus Tamakugu* Masamune et Syozi (Masamune et Fukuyama, no. 657! in Herb. Taiwan Univ.). *M. Tamakugu*, described from Hainan Is. is nothing else than a small plant of *Cyperus compactus*. This species comparatively varies in the size of its vegetative parts and in the length of spikelets as well as in other large-sized tropical species of *Cyperus*.

Cyperus szechuanensis T. Koyama, spec. nova e vicinia *Cyperī nivei* Retzius a quo habitu grandiore robustioreque, foliis bracteisque longioribus, spiculis latioribus distincte recedit, et abs *Cypero fulvo-albescente* T. Koyama foliis culmisque multo brevioribus, spiculis numerosioribus minus florentibus, squamis minoribus conspicue carinatis praecipue distat. Sectio *Platystachyi* Kunth. (Fig. 10)

Herba perennis mediocris. Rhizoma breviter decumbens crassum lignosum squamis castaneofuscis obtectum, internodiis brevissimis, radicibus tenuibus sed validis longis. Culmi uniserialiter erecti pauci approximati 15-26 cm alti triquetri 1.5 mm crassi sulcati laeves inferne foliis paucis obsiti et basi bulbosoincrassati, bulbis oblongo-ovoideis 10-15 mm longis circiter 5 mm crassis vaginis foliorum mortuorum laminatis vel spathaceis castaneofuscis vel castaneobrunneis haud fibrososolutis arcte vestitis. Folia normalia pluria usque 8 linearia mollicula culmo conspicue superantia 15-40 cm longa unico-

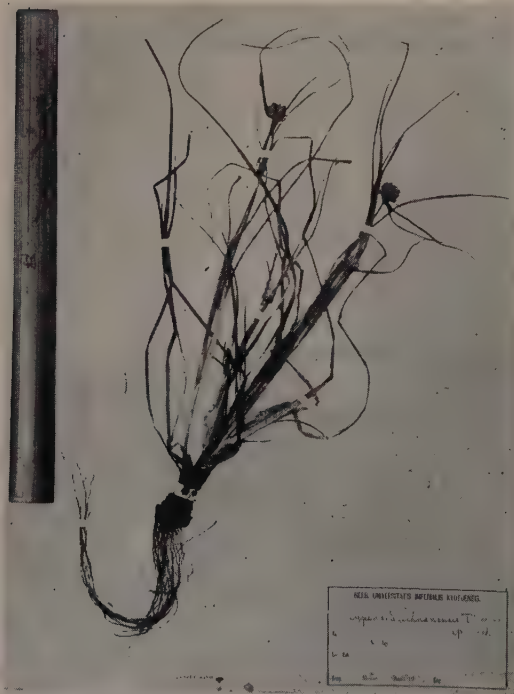


Fig. 10. *Cyperus szechuanensis*
T. Koyama (type).

stata planiuscula margine scabriuscula plus minus involuta apice longe acuminata basi in vaginas longas antice hyalinas culmum arcte circumdantes haud attenuata, ligula nulla. Bractee 2-3 foliaceae patentissimae demum reflexae inferiores 2 quam inflorescentia multo longiores ad 9 cm longae. Anthela simplex capitatocontracta; capitulum globosum circiter 15 mm in diametro. Spiculae 18-30 radiatim dispositae ovatoellipticae vel ellipticae compressae 5-9 mm longae (2-) 3-4.5 mm latae distiche 6-12-florae, rhachi vix compressa anguste alata, alis membranaceis linearibus fuscescentibus. Squamae spissae patentes ascendentes plus minus incurvae late ovatae 3.8-4.5 mm longae chartaceomembranaceae albofulvescentes intus dilute cinnamomeae latere utroque obsoletissime 4-7-nerviae margine late albohyalinae involucentes

apice obtusiusculae mucronatae saepe subtridentulae, costa prominente 1 (-3)-nervosa apice mucronem rectem formante. Antherae 3 circiter 1.5 mm longae, connectivo apice obtuso. Nux immatura, stylo longo plus quam 1 mm longo, stigmatibus 3 breviusculis.

Szechuan: (F. T. Wang, no. 20000D!—Holotype in KYO).

Fimbristylis stolonifera C. B. Clarke in Hook. f., Flor. Brit. India 6: 637 (1893) et in Journ. Linn. Soc. 36: 245 (1903); Ohwi et T. Koyama in Bull. Nat. Sci. Mus. n. s. 3: 29, t. 5, f. A-G (1956).

Kiangsu: Suchow, Mt. Tienping-shan (H. Migo!—TI; TNS)—Yünnan, Indo-China, India.

From the achene character this species is placed in the section *Dichelostylis*, however, well elongated wiry stolons covered with reddish-brown scales and solitary culms are a very rare occurrence in *Fimbristylis*, only found in *F. Pierotii* Miquel, a member of the section *Trichelostylis*, to take a very simple case.

Hypolytrum Ohwianum T. Koyama, spec. nova ex affinitate *Hypolytrii latifolii* L. C. Richard quo nuce multo minore 2.5 mm longa circiter 1 mm lata, squamis orbiculato-ovalibus, foliis vaginis basilaribusque valde glaucis, habitu minore dissimilis est et ab *Hypolytro formosano* Ohwi habitu multo grandiore, squamis latoribus orbiculato-ovalibus satis distinguitur. (Fig. 11)

H. latifolium (non L. C. Richard): C. B. Clarke in Journ. Linn. Soc. 36: 258 (1903), saltem pro parte; E. G. Camus in Lecomte, Flor. Génér. de Indo-Chine 7: 171, f. 22: 2-8 (1912).

Rhizoma breviter repens crassum lignosum squamis rigidis cinereis et cinnamomeorubentibus remote obsitum radices varidas fluvoglaucas pauca^s emittit. Culmus solitarius robustus erectus 35-90 cm altus triqueter obtusangulus striatus 2-3 mm crassus basi 1-2-foliatus et raro sub inflorescentia unifoliatus. Folia multa praeter superiores 2 subradicales, radicales culmum duplo triplove superantia late linearia 10-23 mm lata subrigida glaucoviridia sursum planiuscula versus basin plicata supra bicostata subtus unicostata marginibus, praeter versus apicem spinoso-scaberrima, laevisima apice subabrupte attenuata breviter acuta basi in vaginas 6-17 cm longas cinereocinnamomeas antice fuscohylinas mox irregulariter fissas vix attenuantia; vaginae basilares inferiores aphyllae superiores laminatae cinereocinnamomeae vel fuscatorubentes opacae subcoriaceae. Panicula unica terminalis corymbosa subglobosa bis ter composita 4-6 cm longa 4-8 cm lata subdensa; bracteae inferiores 1-2 (-3) foliaceae, ima inflorescentiam multo superans circiter 1 cm lata, sequens cum inflorescentia subaequans, ceterae subsquamiformes, omnes evaginantes; prophylla ochreiformia 3-7 mm longa herbacea versus apicem fuscotincta dorso bicostata apice oblique secta; rami a unica axila bracteae bini ternive inferiores rectangulariter divergentes superiores oblique patentes rigidi 9-27 mm longi ad angulos sparse hispidi apici racemo vel panicula secundaria constructi (4-11-spiculigeri); ramuli a ochrea fusca glumacea orti etiam rectangulariter divergentes minute hispiduli; bracteolae setaceae interdum subnullae; spiculae solitariae maturitate fere globosae 3-7 mm longae 2.5-6 mm crassae spisse pluriflorae. Squamae imbricatim dispositae orbiculato-ovales vel orbiculato-obovatae naviculares 1.3-1.5 mm longae circiter 1.2 mm latae sordide fuscae et brunneostriolatae opacae durae glabrae apice rotundatae medio unicostatae. Squamellae (prophylla) 2 late ellipticae 1.2 mm longae hyalinae rubrofuscae apice rotundatae



Fig. 11. *Hypolytrum Ohwianum* T. Koyama.

margine anguste scariosae clarius coloratae. Stamina 2; antherae 1 mm longae. Nux maturitate divaricata vel patula orbiculato-ovata 1.8-2.2 mm longa 1.3-1.5 mm lata biconvexa valde inflata longitudinaliter grosse paucirugosa, infra medium obscurius fusca, margine angustissime dure alatocostata, basi rotundatocontracta brevistipitata, sursum olivaceoflava subabrupte contracta in rostrum rectum conicum subbreve circiter 1/2 mm longum; stylus subnullus, stigmatibus 2, 1.5 mm longis dein deciduis.

Hainan: Yeungling-shan (S. K. Lau, no. 197!—TI; KYO); Kwantung (KYO!); Tonkin: Laokay (B. Hayata, sin. num.!—Holotype in TI).

This new species which occurs in Southeastern China and Northern Indo-China, has hitherto been included in *H. latifolium* L. C. Richard which is widely extending in the tropical regions from India to Australia and to Micronesia. I separate this species from the Indian and Micronesian *H. latifolium* L. C. Richard by its generally smaller floral parts and very glaucous vegetative parts especially seen in its basal sheaths. The epithet is dedicated to Dr. J. Ohwi who was first of opinion that the South Chinese plants might be distinguished from *H. latifolium* L. C. Richard, when he distinguished *H. formosanum* Ohwi from it.

Besides the specimens cited in the present paper, several new entities of *Eleocharis*, *Fimbristylis* and *Carex* have recently been detected in another Central Chinese collection of Cyperaceae. The collection was made by Dr. Hisao Migo from 1933 to 1942, when he had been in the Shanghai Science Institute. It is regrettable that the majority of this collection was not brought to Japan owing to the last World War. In the Bulletin of the National Science Museum, N. S., Vol. 3, pp. 18-32, Dr. Ohwi and I have given an account of the cyperaceous specimens of the collection in which several nomenclatorial changes were made and the new plants mentioned were validated.

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Fig. 11. *Hypolytrum Ohwianum* T. Koyama:—

AA. habit; B, C, D. parts of leaf, upper surface of middle part (B), upper surface of apical part (C) and lower surface of apex (D); E. part of culm; F. dorsal view of prophyll; G. ventral view of the apex of do.; H. flowering spikelet without stamens; I. apical part of branch with fruiting spikelets; J. flower with squamellae; K. scale; L. ventral view of squamellae with withered filaments; M. achene; N. floral diagram. (from various specimens cited).

Variations in *Sphagnum junghuhnianum* var. *pseudomolle* Warnst. and the Status of *Sphagnum kiiense* Warnst.*

By

Hyoji SUZUKI

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Introduction

Sphagnum junghuhnianum ranging along the southern part of Asia includes three known varieties, namely, vars. *typicum*, *gedeanum* and *pseudomolle* which have been recorded from Japan with the exception of the second. The three varieties were originally described as independent species, but Warnstorf (1911) united them under *Sphagnum junghuhnianum* because of the close resemblance in the shape and structure of their branch-leaves. However, he mentioned the differences in shape, structure and size of stem-leaf as the more important characteristics which could serve to distinguish the varieties. Cardot (1906) had already mentioned that two forms of stem-leaves were observed on the same stem in some specimens of var. *pseudomolle* from Taitum, Formosa.

Having studied numerous specimens (above 180 packets) from Japan, which are presumed to belong to this species, the writer has concluded that the occurrence of var. *typicum* in Japan is very doubtful and that *S. kiiense* is possibly comparable to var. *pseudomolle* and has reached the further conclusion that var. *pseudomolle* is to be treated as an independent species or at least as a subspecies.

Observations and Considerations

1. Variations in the external characteristics and others. External habits are produced by the combination of several characteristics, mainly the color, texture, and density of branch-fascicles and the length or direction of the branch, as well as the shape or arrangements of their leaves.

The color of this plant varies from pale green to yellowish brown. Generally speaking, the plants growing in the sheltered situation are usually greenish, while the ones in the open situation are yellowish brown, and they turn to a dark color in the cold season. Such a tendency is observed not only within a small patch, but also in one individual, and the brownish color predominates at the top of the same plant. The deeply colored plants have occasional tint of light pink.

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Whether the branch-fascicles are dense or not depends entirely upon the water conditions in the substratum on which the plant is growing. In the wet place they have usually long branches arranged in loose fascicles on the stem, so whether they are in sheltered or exposed situations is of no consequence. Regarding the length of branches, the same tendency is occasionally observed.

The leaves are, more or less strongly squarrose at the basal part of the branch, spreading in the middle part and imbricate near the apex, but, plants growing in the sheltered place have usually strongly squarrose leaves on almost the basal half of the branch, while those of the exposed place are predominated by the imbricated leaves.

Both the shape and size of the branch-leaf are very variable, especially in size, depending upon the environments in which the plants are growing. The structure of the leaf shows some variations; however, this is so insignificant and precludes the necessity of distinguishing such variations.

2. Variations in the structure of stem-leaves. Warnstorf (1904, '11) characterized the structure of stem-leaves of var. *pseudomolle* as follows:

“entweder beiderseits porenlos oder auf der Innenfläche des Blattes mit undeutlich begrenzten Membranlücken.”

However, in the material from Japan and Formosa, the writer was unable to recognize any stem-leaf which did not have any kinds of perforations on both surfaces, but there are constantly encountered some kinds of perforations varying from large half-round or elliptic pores to membrane-gaps, at least on the inner surface. When the large membrane-gaps occupy nearly the entire surface of the cell and without fibers on both surfaces, even when the leaves are tinged with stain, there is a possibility of erroneously concluding that perforations are not existent.

In the stem-leaves of the Japanese specimens examined, the following five types are classifiable on the structure mainly characterized by the perforation and fibrillation on both surfaces of the leaf.

- O-type:** Hyaline cells of both surfaces without fibers, the outer surface without pores and the membranes of the inner surface almost completely resorbed.
- A-type:** Upper part of the leaf more or less strongly fibrous, the outer surface completely without pores, the inner surface with numerous round pores or large membrane-gaps.
- B-type:** Upper part of the leaf with numerous fibers, outer surface usually with a few round pores at the upper part of the leaf, the inner surface provided with pores as in A-type.
- C-type:** Both surfaces provided with numerous fibers almost up to the leaf-base, outer surface with more numerous half-round to elliptic pores arranged in a series near the commissure, the shape of the leaf usually triangular with a broad base, although its structure strongly resembles the branch-leaf in structure.
- D-type:** Closely resembling the branch-leaf in shape and structure, usually with a narrow base.

As many transitional forms are encountered, it is occasionally difficult to determine to which type they should be assigned, except those of the O- and D-types. Generally speaking, O-, A- and B-types can be considered to belong to anisophyllous form, while C- and D-types to the subisophyllous or isophyllous form. It is very interesting to note that the occurrence of these types is most irregular, not only in specimens from the same area, but also on the same stem as was recorded by Cardot, also to be noticed is the fact that various combinations of different types of the leaf can be recognized. Table 1 graphically shows the occurrence of each type and their combinations on the same stem, as observed in the specimens from four different districts of Japan. Considerable difference can be recognized in the occurrence of the isophyllous stem-leaf from these four districts. It might be concluded from this table that the greater part of specimens from Yakushima Island and the Kii Peninsula belong to an anisophyllous form, while more than one half of the specimens from Shikoku and Miyajima Island belong to a subisophyllous or isophyllous form.

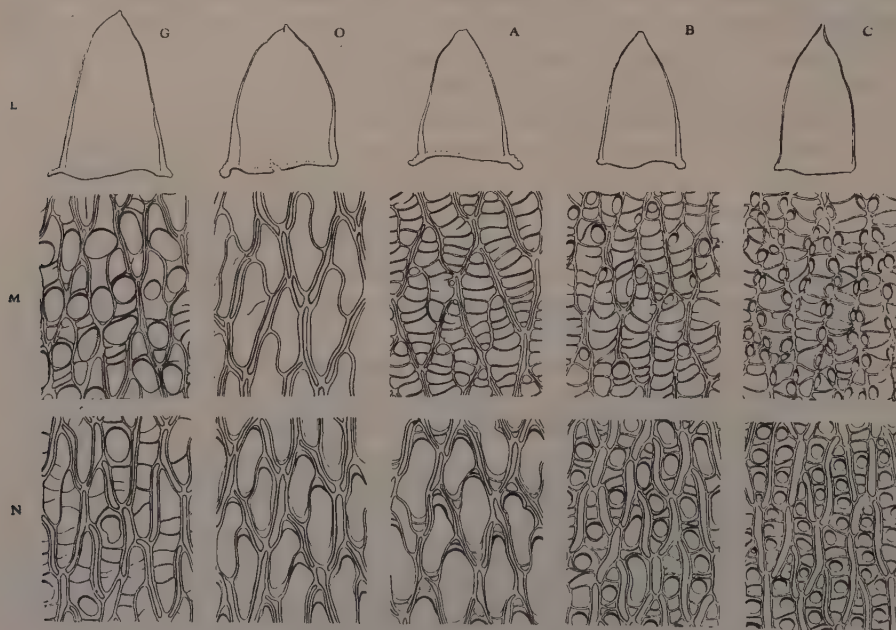


Fig. 1. Various types of stem-leaf classified by perforation and fibrillation on both surfaces.

L. Stem-leaves $\times 12$; M. Cells on the outer surface $\times 157$; N. Cells on the inner surface $\times 157$; All cells were drawn from the upper middle part of the leaf, G-type was drawn from a specimen of *S. junghuhnianum* var. *gedeanum* from East Java (Exsiccata, Fleischer no. 501), O-, A-, B- and C-types were drawn from the specimens from Yakushima Isl. H.S. nos. 3791, 3888, 3892 and 3884 respectively.

From current descriptions it is evident that the perforation of stem-leaves of both var. *typicum* and var. *gedeanum* are usually more prominent on the outer surface than on the inner surface, as is demonstrated by the G-type in

Table 1. Occurrence of the types of stem-leaf in four districts.

Districts	Number of packets	Combinations of types of stem-leaf										Ratio	
		anisophyllous				isophyllous						(aniso. : iso.)	
		O	A	B	AB	AC	BC	OABC	ABC	C	CD	Number	Percentage
Yakushima Isl.	23	2	10	3	1	1	0	0	3	1	2	16 : 7	70 : 30%
Kii Peninsula	21	0	10	4	0	0	0	0	6	1	0	14 : 7	66 : 33 "
Shikoku	22	0	6	2	1	1	2	1	4	5	0	9 : 13	41 : 59 "
Miyajima Isl.	26	0	5	5	0	1	0	0	14	0	1	10 : 16	38 : 62 "

fig. 1. This constitutes an essential characteristic which serves to distinguish these varieties from var. *pseudomolle* which is exactly opposite in this point. The large membrane-gaps have not previously been recognized correctly on the outer surface of the stem-leaf, even in the isophyllous form taken from Japan. The writer believes that records of var. *typicum* from Japan were perhaps a mistake which was due especially to misjudging the subisophyllous or isophyllous form. All the specimens from Japan which exhibit these characters are best included under var. *pseudomolle*.

3. Variations in the shape and size of stem-leaves. There are four populations of this moss in a small area of Miyajima Island. Two of them are situated along a small valley and the two others are on the steep rock-walls which belong to the same slope. One of the former is a small patch hanging on a shady cliff near the Shiraito Waterfall at the altitude of about 80 meters. The other is larger than the former and spreads on an open, wet, granite outcrop, so-called Makuiwa, at the altitude of 280 m. On the other hand, one of the latter is located at the altitude of about 500 m and the other at about 550 m on the west slope of Komagabayashi Hill.

Various habits of the plant were observed in these communities corresponding to the varied edaphic and atmospheric conditions which are to be found there. The writer has carefully collected numerous plants from these plots, and selected 32 packets representing various habits out of those. He removed from ten to thirty stem-leaves from a representative plant of each packet and examined their shape and size or structure.

Fig. 2 shows the distribution of size of 675 stem-leaves. In this figure the shape of the leaf also may be suggested by means of the oblique lines denoting the rate of the length to the width, namely lines **a**, **b**, **c** and **d** indicate that the length is 1.2, 1.5, 2.0 and 2.5 times as long as the width respectively.

It is very clear in this figure that there is a great variation in size, that is, the length varies from 1.04 to 1.80 mm and the width varies from 0.64 to 1.20 mm, the majority belongs to the size of 1.32~1.60×0.68~0.92 mm. As to the shape of the leaf, it is evident that the majority (534=79%, see also Table 3) has leaves of the median type (length 1.5-2.0 times as long as the width) and a part of the remainder (84=12%) has the long type (length 2.0 and more

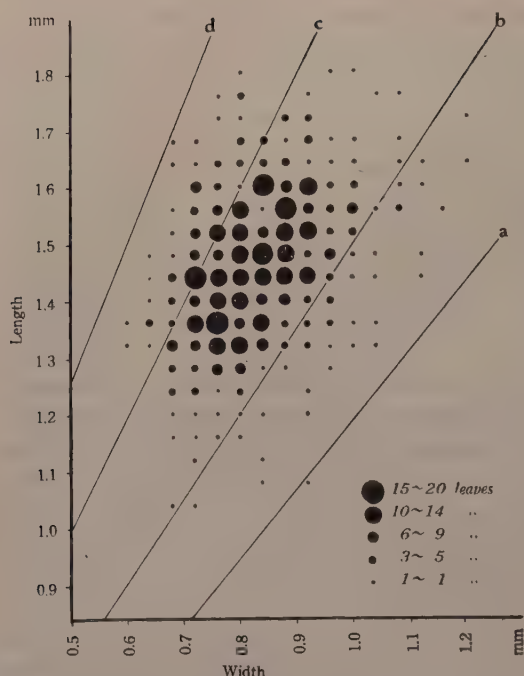


Fig. 2. Distribution of the size of 675 stem-leaves from samples collected from Miyajima Island.

times as long as width) and another part of that (56=8%) has short ones (length 1.2 and less times as long as the width).

At this point, it should be recalled that one of the key point in distinguishing var. *gedeanum* from var. *typicum* is the shorter and broader stem-leaf of the former. However, it appears that the shape and size of the stem-leaf of this plant is not an important characteristic upon which to classify its varieties. As a matter of fact, the shape of stem-leaves observed in a specimen of var. *gedeanum* was rather longer and narrower (see Fig. 1. G-type) than the figure in the illustrations by Dozy & Molkenboer (1885). It seems best to treat var. *typicum* and var. *gedeanum* as the same taxonomic unit, for such differences as may be recognized be-

tween these two varieties may be covered by the amplitude of variation observed in material from Miyajima Island.

The same method of summarization used for Fig. 2 is used for Fig. 3, for the samples from Yakushima Island, where this moss grows at the altitudes of 1300 to 1760 m. From these figures it can be observed that there exists a great variation in the size and shape of the stem-leaf of this plant even when samples are collected in a restricted area.

Table 2. Variation in the size of stem-leaves from four districts.

Districts	Total of examined leaves	Shape of stem-leaf Number (percentage)			Length mm			Width mm		
		long	median	short	min.	max.	mean	min.	max.	mean
Yakushima Isl.	256	24 (10)	167 (65)	66 (26)	0.88	1.80	1.24	0.56	1.12	0.76
Kii Peninsula	267	34 (13)	188 (70)	45 (17)	1.04	1.80	1.48	0.60	1.12	0.80
Shikoku	329	91 (28)	198 (60)	40 (12)	1.04	1.88	1.56	0.56	1.12	0.80
Miyajima Isl.	675	51 (9)	466 (78)	78 (13)	1.04	1.80	1.44	0.64	1.20	0.80

Table 2 is a summary on the shape and size of stem-leaf of specimens examined from four different districts of Japan. It can be concluded from this table that specimens from Shikoku district generally have slender leaves while those from Yakushima Island have short leaves. The stem-leaf of this

plant shows somewhat of a dispersal in its size and shape as well as the occurrence of its types, in accordance with the geographical locations in which it grows.

The writer could not find any kind of cline in the variation of the stem-leaf, and then, even though there are some kinds of difference in the populations, it was usually less important than the difference recognized in the results of the examination of the samples from Miyajima Island. It may be true that all the plants from Japan considered as belonging to *S. junghuhnianum* really belong to a single taxon; strictly speaking, to var. *pseudomolle*.

4. On some authentic specimens from Formosa. Warnstorf (1904) based his description of *S. pseudomolle* upon a specimen from Formosa (Faurie no. 48, May 7, 1903, Taitum, 1200 m). An isotype of this species and some authentic specimens which were collected by Faurie and were referred by Cardot as belonging to *S. jungghuhnianum* or *S. pseudomolle* are preserved in the herbarium of Kyoto University. Through the courtesy of Prof. S Kitamura of Kyoto University the writer was afforded the opportunity of examining these specimens. The result of the examination of these specimens for structure of stem-leaves is shown in the following table.

Table 3. Occurrence of the types of stem-leaf in the specimens identified by Warnstorf or cited by Cardot.

Identified names	<i>S. junghuhnianum</i>	<i>S. pseudomolle</i>
Faurie's number	40 215 216 217 218	48 219 221
Observed leaf-types	A A ABC D A	A AB AB

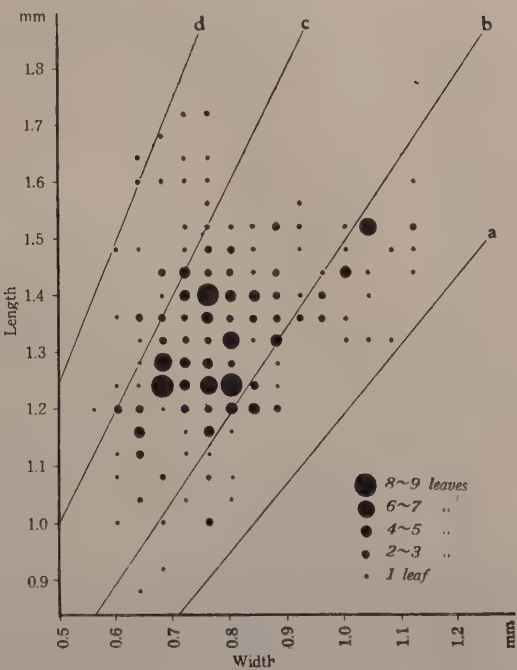


Fig. 3. Distribution of the size of 256 stem-leaves examined on the specimens from Yakushima Island.

Not only any novel type of stem-leaf was not observed, but also no essential differences in occurrence of the leaf-types are to be recognized in the two groups. Fig. 4 shows the results of the examination of the size and shape of a stem-leaf of the same specimens.

More or less distinct dispersal of variation is indicated in this figure, but it is very doubtful if there

were any essential difference among them as was expected. A comparison of the figures 2, 3 and 4 clearly shows that the dispersal of the stem-leaf noted among the specimens from Formosa represented in Faurie's collections is safely to be confuted.

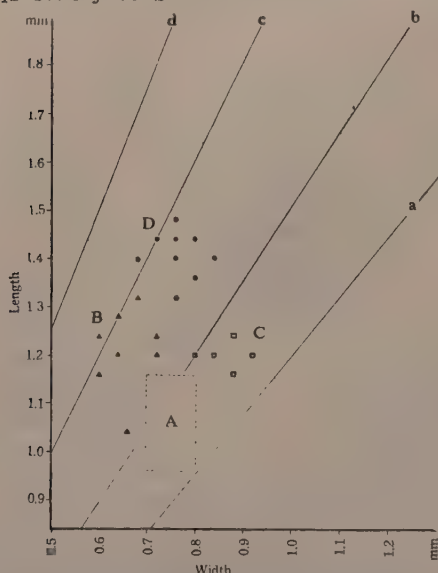


Fig. 4. Variations in the size and shape of a stem-leaf in some authentic specimens. A. from the original description of *S. pseudomolle*; B. from the isotype of *S. pseudomolle* (Faurie no. 48); C. from one of the specimens designated as *S. pseudomolle* by Cardot (Faurie no. 219); D. from one of the specimens designated as *S. junghuhnianum* by Cardot (Faurie no. 216).

5. On *Sphagnum kiiense* Warnst.

This was first described by Warnstorf (1911) on a specimen collected by Sh. Okamura in the Kii Province (Kii Peninsula). The original description of the species brings to our mind a similarity to var. *pseudomolle*, the subject of this paper. There are no definite characteristics in his description which could serve to distinguish *S. kiiense* from var. *pseudomolle*. In *Sphagnologia universalis* Warnstorf assigned this plant to the *deltoideo-lingulata* group (Subser. 3) and *S. junghuhnianum* to the *deltoidea* group (Subser. 2) according to the shape of stem-leaf. But, he reported at the same time that stem-leaves of var. *pseudomolle* are mostly triangular lingulate. Thus the essential difference between *S. kiiense* and var. *pseudomolle* becomes very vague.

In the herbarium of the National Science Museum in Tokyo, there is a packet of specimen of *S. kiiense* with the following data: Prov. Kii, Yomura, July 8, 1911, Leg. Otokubo, det. Sh. Okamura, **Co-type**.

However, it is clear that this specimen is not the co-type of this plant, judging from the citation (viz. Japan: Prov. Kii, Shutai Okamura, no. 23, XI 1906) added to the original description by Warnstorf. Another important factor that it is not the co-type is suggested from the fact that sexual organs were not treated in the original description, while many brilliant large perichaetial leaves were observed in this so-called co-type specimen.

At any rate, the writer was able to examine this specimen through the courtesy of Dr. Y. Kobayashi in the National Science Museum, and he has obtained the following results which offer us nothing of fundamental difference from var. *pseudomolle*.

Although this specimen is not the co-type of *S. kiiense*, and though the writer has been unable to examine the type, of all the numerous specimens from Kii Peninsula examined by the writer there is none which could be

Table 4. Results obtained from examination of the so-called co-type specimen of *S. kiiense*.

	Stem-leaf			Perichaetial leaf
	Length	Width	Leaf-type	
On a sterile stem	1.60-1.88	0.70-1.04	ABC	Exactly coincide with the description of <i>S. junghuhnianum</i> in their shape, size and structure
On a fertile stem	1.44-1.55	0.72-1.04	OA	

identified as *S. kiiense*, independent from var. *pseudomolle*. For these reasons, it is concluded that *S. kiiense* is possibly a synonym of var. *pseudomolle*.

6. On the geographical distribution. The known ranges of the varieties of *S. junghuhnianum* are as follows:

var. *typicum* : Java, Celebes, Philippines, Formosa and Japan.

var. *gedeanum* : Java, Moluccas, New Guinea and Celebes.

var. *pseudomolle* : Himalaya, China, Formosa and Japan.

Formosa and Japan should be omitted from the range of var. *typicum* from the results of studies embodied herein. A striking fact can now be seen that the range of var. *pseudomolle* is limited to the northern part of the range of *S. junghuhnianum*. Moreover, if the presence of var. *typicum* in the Philippines were based on the misjudgement of var. *pseudomolle* like the case of the specimens from Japan and Formosa, it may be said that *S. junghuhnianum* (consisting of vars. *typicum* and *gedeanum*) and *S. pseudomolle* (var. *pseudomolle*) have different ranges respectively. This is the reason why the writer strongly urges a revision of species from the Philippines.

Conclusion

A study of the variation of the important characters which serve to distinguish the varieties of *Sphagnum junghuhnianum* Dozy & Molkenboer (1854) was investigated by the writer and has lead him to the conclusion that the species includes two different forms which merit a taxonomic recognition. Further he concludes that all specimens from Japan and Formosa formerly considered to represent this species comprise one of these forms, namely *S. pseudomolle* originally described by Warnstorf (1906) on Formosan specimens. *S. kiiense* established by Warnstorf (1911) on a specimen collected in the Province of Kii is considered as representing nothing but *S. pseudomolle*.

The features of variation and geographical distribution as well as the close affinities in the sexual organ or branch-leaves were considered. These forms seem to be recognizable as subspecies. They can be distinguished from each other by the following characteristics.

Perforation of stem-leaf constantly more prominent on the outer surface than on the inner surfacesubsp. *junghuhnianum*

Perforation of stem-leaf constantly more prominent on the inner surface than on the outer surface..... subsp. *pseudomolle*

1. ***Sphagnum junghuhnianum*** Dozy & Molkenb. subsp. ***junghuhnianum***
Sphagnum junghuhianum Dozy & Molkenb. Verhandel. Kon. Akad. Wetensch. (1854); Bryol. jav. 1:27. *t.* 18 (1855); Warnst. Hedwigia 29:198. *t.* 5. *f.* 11a, 11b & *t.* 7. *f.* 10 (1890), Engler-Prantl, Nat. Pfl.-fam. 3:259 (1901); Fleischer, Musc. Flora Buitenz. 1:8 (1904); Paris, Index bryol. 5:282. (1905); Paul, Engler-Prantl. Nat. Pfl.-fam. ed. 2. 10:115 (1924).
Sphagnum gedeanum Dozy & Molkenb. Verhandel. Kon. Akad. Wetensch. (1854); Bryol. jav. 1:28, *t.* 19 (1855); Warnst. Hedwigia 29:99, *t.* 5. *f.* 12a, 12b, & *t.* 7. *f.* 9 (1890); Fleischer, Musc. Flora Buitenz. 1:7 (1904); Paris, Index Bryol. 5:278 (1905).
Sphagnum thomsonii C. Müll. Linnaea 545 (1874).
Sphagnum junghuhnianum Dozy & Molkenb. var. *typicum* Warnst. Sphagnol. univ. 116 (1911); Fleischer, Musc. Flora Buitenz. 4:1631 (1922).
Sphagnum junghuhnianum Dozy & Molkenb. var. *gedeanum* Warnst. Sphagnol. univ. 116 (1911); Fleischer, Musc. Flora Buitenz. 4:1631 (1922).
2. ***Sphagnum junghuhnianum*** Dozy & Molkenb. subsp. ***pseudomolle*** (Warnst.) H. Suzuki, *stat. nov.* (Fig. 1, O~C-types & Fig. 5).
Sphagnum pseudomolle Warnst. Beih. Bot. Centralbl. 16:247 (1904); Cardot, ditto 19:89 (1906).
Sphagnum kiiense Warnst. Sphagnol. univ. 82 (1911); Paul, Engler-Prantl, Nat. Pfl.-fam. ed. 2. 10:114 (1924). *syn. nov.*
Sphagnum teres Aongstr. (?) Sakurai, Bot. Mag. Tokyo 47:345 (1933).
Plants rather large, often somewhat delicate, 5-10 cm high or higher, usually soft, pale green, yellowish green to yellowish brown or dirty brown, occasionally bearing light pink at the coma, without lustre or with more or less lustre. Wood-cylinder dirty brown to reddish brown; cortical cells of the stem in 3 layers, occasionally in 2 or 4 in some part of it, often on the half of periphery larger than the others, the walls thin, without fibrils, on the outer surface short or long quadrilateral, usually without pores, often with round or half-round thinnings of the wall near the upper end of the cell.
Stem-leaves fairly large, isoscleres-triangular to triangular-lingulate, occasionally almost equilateral triangular, (0.88-) 1.20-1.60 (-1.80) mm long and (0.56-) 0.70-0.90 (-1.20) mm wide at the base, narrowly truncate or obtusely truncate at the apex bearing 5-10 minute teeth, the margin often undulate, a little involute toward the apex and sometimes bent backward; the border entire, of 2-3 (-6) rows of narrow cells with pitted walls, not or slightly broadenning toward the base; hyaline cells rhombic to rhomboidal, with simple or compound divisions on almost all of the leaf surface, rarely without divisions in the apical part, with or without fibrils on the upper half, occasionally fibrous up to the base of the leaf; on the inner surface the membrane almost completely resorbed in the fiberless forms and commonly with numerous round pores in the fibrous forms, on the outer surface without pores in the former and with a few to numerous half elliptic pores along

the commissures in the latter, constantly more porous on the inner surface than the outer; the auricles rather small.

Branches in fascicles of 3, often 4, 2 of which spreading, short or slender, sometimes large and fairly thickened, up to 21 mm long, their cortical cells in a layer, retort-cells with a pore and with inconspicuous neck, often 2 and rarely 3 cells in a row; branch-leaves usually imbricate, often squarrose on the basal half of the branch, ovate to ovate-lanceolate, (1.28-) 1.40-1.80 (-2.25) mm long and (0.60-) 0.70-0.90 (-1.20) mm wide, strongly concave, the margin somewhat undulate, strongly involute near the apex which is obtusely truncate with 5-7 teeth, the border entire, of 1-3 rows of narrow cells; hyaline cells fibrillose, vermicular near the apex, 8-13 times as long as the width, 6-8 times and broader in the middle part and 4-6 times or still broader near the base; on the inner surface with large round pores along the commissures in narrow side regions, the pores rarely somewhat remote from the commissures, besides with small pores at both ends of cells near the apical part, on the outer surface with numerous elliptic to half elliptic pores along the commissures, the pores usually enlarged toward the base, besides sometimes with large round pores on the walls of the cells in the middle side regions; the leaves of the pendent branch usually smaller than the normal branch-leaf, with more numerous pores on both surfaces. Chlorophyll-cells more or less broad triangular to trapezoid in section with broad exposure on the outer surface, exposed on the inner surface or on both surfaces.

Polyoecious; antheridia in catkins on spreading branches, antheridial leaves somewhat tinged with brown, slightly differentiated, round-ovate to ovate shorter than the normal branch-leaf, 0.93-1.20 mm long and 0.60-0.70 mm wide, truncated at the apex bearing 3-5 teeth, strongly concave, somewhat constricted near the base, the margin involute, the border entire, of 1-2 rows of narrow cells, occasionally more or less serrulate near the apex; hyaline cells of the lower part without pores on both surfaces, often lacking the fibrils in a few cells near the base, perforation in the remainder similar to the normal branch-leaf; fruiting branches short or long, erect, perichaetial leaves up to 15 in number with brilliant lustre when dry, ovate-oblong to oblong, innermost one largest, up to 5 mm long and 3 mm wide, slightly emarginate at the apex, composed of two kinds of cells in a greater part of the leaf excepting basal part which are composed of narrow vermicular cells with pitted walls, the border narrow, of 2-5 rows of narrow cells, hyaline cells broadly rhombic, narrowing below, with simple or compound divisions on the whole surface, usually without pores and fibrils, often with irregularly round pores or membrane-gaps on the inner surface, rarely with weak fibrils in a few cells on the upper part of the leaf; capsule dark brown, sphaerical, 15.0-17.5 mm in diameter; spores pale yellow, slightly brown in mass, 21.0-23.0 μ in diameter, minutely granular roughened, oil body indistinct.

Specim. exam. **Honshu**: Prov. Echigo, *Tsugawa** Higashikanbara-gun, *Tsugawa*-

* The geographical names printed in italic in this paragraph indicate the names of topographical maps (1/50,000) (viz. one of Horikawa's geoquadrats) and they comprise the localities of the examined specimens.

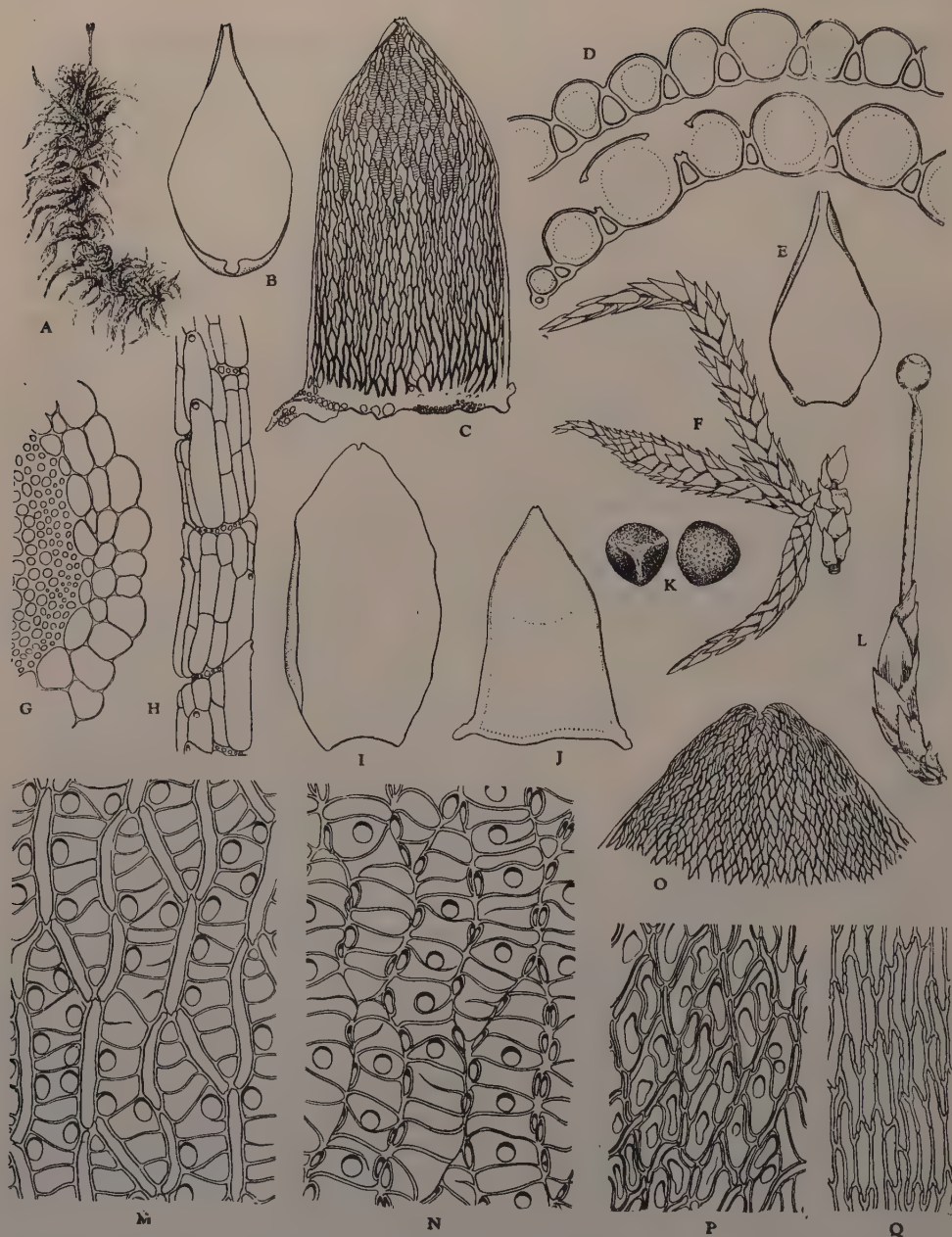


Fig. 5. *Sphagnum junghuhnianum* subsp. *pseudomolle* (Warnst.)
 A. Fruiting plants $\times 3/4$; B. Branch-leaf $\times 19$; C. Areolation of stem-leaf $\times 38$; D. Cross sections of branch-leaf $\times 335$; E. Antheridial leaf $\times 19$; F. Branch-fascicle bearing an antheridial catkin $\times 5/2$; G. Cross section of stem $\times 110$; H. Part of denuded branch $\times 58$; I. Perichaetial leaf $\times 9$; J. Stem-leaf $\times 19$; K. Spores $\times 335$; L. Fruiting branch $\times 5/2$; M. Inner surface of marginal cells of branch-leaf $\times 235$; N. Outer surface of ditto $\times 235$; O. Apical part of perichaetial leaf $\times 58$; P. Inner surface of upper part of perichaetial leaf $\times 110$; Q. Cells from basal part of ditto $\times 110$.

machi, Kirinzan (Y. Ikegami, Oct. 9, 1950): Prov. Mino, *Kanayama*, Mugi-gun, Nakanohomura, Wakaguri, 125 m (T. Inoue, Feb. 26, 1952, H.S.** Oct. 15, 1953); Prov. Owari, *Gifu*, Niwa-gun, Inuyama-machi (H. Fukuhara, July 31, 1949); Prov. Mikawa, *Mikawaono*, Kitashidara-gun, Miwa-mura, Kawai (N. Takaki, Aug. 21, 1947), *Taguchi*, ibid. Chichii-wakyo, 140 m (H. Ando, Nov. 1, 1954), ibid. Kamebuchidani (M. Tagawa, Sept. 21, 1953); Prov. Tanba, *Yotsuya*, Kitakuwada-gun, Chii-mura, Ashiu Experimental Forest of the Kyoto University (M. Tagawa, Aug. 27, 1951, T. Nakajima, Aug. 27, 1951, c. fr., H. Muroi, June 1951, T. Nakajima May 3, 1954), *Sonobe*, Taki-gun, Oota-mura, Koganedake, 600 m (T. Nakajima, Oct. 24, 1954), ibid. 650 m (T. Nakajima, Oct. 24, 1954); Prov. Yamashiro, *Kitakomatsu*, Kyoto-shi, Sakyo-ku, Daihiyama (M. Hiroe, Sept. 24, 1951); Prov. Ise, *Oodaigahara*, Taki-gun, Oosugi-mura, Sanzukocho 1200-1500 m, on limestone (T. Kodama, Aug. 17, 1951, ibid. Shokudodani (N. Takaki, Aug. 3, 1948), ibid. Oosugidani (N. Takaki, Mar. 13, 1949), *Niseura*, Doai-gun, Hobara-mura, Oshibuchi, 150 m (T. Kodama, Mar. 30, 1954); Prov. Yamato, *Yoshinoyama*, Yoshino-gun, Kawakami-mura, Ootaki, (S. Terao, July 3, 1948, c. fr.), *Oodaigahara*, ibid. *Oodaigahara* (S. Terao, Aug. 4, 1947, c. fr.), ibid. Honsawagawa, Shirakuradanideai (T. Nakajima, Aug. 21, 1954), ibid. Shionoha (T. Naka-



Fig. 6. Range of *Sphagnum junghuhnianum* subsp. *pseudomolle* in Japan. Black dots indicate the position of the geoquadrats in which the localities of examined specimens are situated.

jima, Aug. 21, 1954) ibid. Ikadaba—Goshikiyu, 600-650 m (H.S. July 22, 1949, c. fr.), *Shakagadake*, Yoshino-gun, Shimokitayama-mura, Maeoni—Maioniguchi, 280 m (S. Nakanishi, Oct. 19, 1952), *Totsugawa*, ibid. Shimokitayama-mura—Gaidoura (H. Ando, Oct. 25, 1951), ibid. Uramuki—Gyosendake, 1100 m (H. Ando, Oct. 25, 1951), ibid. Totsugawa-mura, Kumagai (H. Ando, Oct. 26, 1951); Prov. Kii, *Kooyasan*, Itsu-gun, Kooyasan-machi, Yatate-Daimon (T. Nakajima, Nov. 3, 1951), *Kurisugawa*, Nishimuro-gun, Futakawa-mura, Ogushi (Y. Ikegami, Jan. 7, 1941), *Tanabe*, ibid. Miyasato-mura, Oouchigawa (N. Kasamatsu, Aug. 3, 1950), Prov. Harima, *Yamasaki*, Shisso-gun, Tomisu-mura, Minago (Y. Tatebe, Apr. 22, 1951), Prov. Iwami, *Tsuwano*, Katari-gun, Asakura-mura, Tadeno (S. Saito, Aug. 1952), Prov. Aki, *Kabe*, Asa-gun, Miiri-mura, Nabarakyo, 300 m (H.S. June 1, 1947),

** H.S. and T.Y. preceding the date indicate the collectors H. Suzuki and T. Yamana respectively.

Itsukushima, Saheki-gun, Miyajima-machi (Y. Ikoma, May 5, 1940), *ibid.* Shiraito Water-fall, 80 m (H.S. May 7, 1948, May 14, 1950), *ibid.* Makuiwa, 280 m (H.S. Feb. 18, 1950), *ibid.* Komagabayashi, 500 m (H.S. May 29, 1948); Prov. Suo, *Ootake*, Kuga-gun, Sakaue-mura, Yasakakyo, 230 m (H.S. June 5, 1955), *ibid.* 240 m (H.S. June 5, 1955 c. fr.); Prov. Nagato, *Chomonkyo*, Abu-gun, Chomonkyo, 210 m (H.S. May 29, 1948).

Shikoku: Prov. Awa, *Wajiki*, Myodo-gun, Sanagochi-mura, Tokuenji (F. Sugimoto, Mar. 12, 1951, T. Hinode, Aug. 18, 1953); Prov. Iyo, *Mishima*, Uma-gun, Kinsha-mura, Orisaka, 350 m (T. Y. June 13, 1950), *Niihama*, Uma-gun, Sekigawa-mura, Ookawa~Koomata, 200 m (T. Y. Sept. 4, 1948), *ibid.* Koomata (S. Yamamoto, June 11, 1951), *ibid.* Mt. Irazu (K. Ochi, July 12, 1948), *ibid.* Besshiyama-mura, Koashidani, 900 m (K. Ochi, Nov. 8, 1942), *ibid.* Mt. Higashiakaishi, 1400-1500 m (T. Y. Aug. 11, 1949), *ibid.* Nakahagi-machi, Koomata (K. Ochi, Aug. 15, 1942, c. fr.), *Yawatahama*, Nishiwa-gun, Futaiwa-mura, Izumi, (K. Inoue, Mar. 1951), *Uwajima*, Uwajima-shi, Daichoji, 300-400 m (T. Y. Oct. 31, 1951), *ibid.* Nametoko, Setsurindaki, 500 m (H. Ando, June 3, 1952), Kitauwa-gun, Kunomura, Gongenyama, 600 m (H. Ando, June 4, 1952), *ibid.* Azame Pass~Uwajima-shi, 450 m (H. Ando, May 17, 1953); Prov. Tosa, *Motoyama*, Nagaoka-gun, Yoshino-mura, Mt. Shiraga, Okushiraga, 870-890 m (T. Y. 27, 1950), *ibid.* Fuyunose, 900 m (H. S. May 15, 1953), *ibid.* Kuwanokawa~Akadaki, 650 m (H. S. May 14, 1953), *ibid.* Kuwanokawa, 450 m (H. S. May 14, 1953), *ibid.* Asemi, 280 m (H. S. May 12, 1953), *ibid.* 180 m (T. Y. Mar. 5, 1950), *ibid.* 170 m (H. S. May 12, 1953), *ibid.* Tai-mura, Nakajima, 280 m (T. Y. Mar. 6, 1950, c. fr.), *ibid.* Motoyama-machi, Kitayama, 580 m (T. Y. June 6, 1948, Sept. 18, 1948), *ibid.* Amatsubo-mura, Koya, 600 m (M. Hara, Apr. 7, 1951, c. fr.), Kami-gun, Akatsuka-mura, Nishimata, 400 m (T. Y. June 6, 1950), *Hibihara*, Tosa-gun, Ookawa-mura, Komugiune, 660 m (T. Y. Mar. 15, 1953), *ibid.* 720 m (T. Y. Mar. 15, 1953), *ibid.* Ookitakawa (T. Y. Mar. 31, 1953), *ibid.* Jizoji-mura, Seto (T. Y. Mar. 30, 1953), *Umaji* Aki-gun, Umaji-mura, Tengumori, 1000 m (T. Y. Mar. 15, 1952), *Shinden*, Takaoka-gun, Higashitsuno-mura, Shinden, 420 m (T. Y. Aug. 24, 1949), *ibid.* 500 m (T. Y. Aug. 23, 1949), *ibid.* Nagasawanotani, 550 m (T. Y. Aug. 18, 1953), *Yuzuhara*, Takaoka-gun, Yuzuhara-mura, Serikawadani, 500 m (T. Y. Aug. 21, 1953).

Kyushu: Prov. Hyuga, *Kumada*, Higashiusuki-gun, Kitakawa-mura, Mt. Ookue, 600 m (T. Amakawa, Mar. 30, 1953), *Shiibamura*, *ibid.* Shiiba-mura, Omai~Kamishiiba, 450 m (H. Ando, Apr. 7, 1953), Prov. Oosumi, *Yakushimatonanbu*, Kumage-gun, Yakushima Isi, Kosugidani~Hananoego, 1000-1600 m (T. Shin, May 17, 1950), *ibid.* 1300 m (H. S. Oct. 19, 1943, K. Negayama, July 28, 1955), *ibid.* 1300-1500 m (H. S. Oct. 19, 1948), *ibid.* 1500-1600 m (H. S. Oct. 19, 1948), *ibid.* Hananoego, 1600 m (Y. Doi, July 28, 1952, H. S. Oct. 19, 1948), *ibid.* Hananoego~Miyanooradake, 1600-1760 m (H. S. Oct. 19, 1948).

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The Vascular Course of Piperales

I. Chloranthaceae

By

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Introduction

Be that as it may, it has been previously concluded that Piperales belongs to one of the primitive group in Angiospermae, mainly on the structure of flower. Since 1949, Swamy and Bailey (1, 2, 3) have been synthetically studying this order, practically Chloranthaceae as the chief subject, observing the vesselless xylem. Judging from their data, however, they have described the vascular courses separately in each organ, and thus have not touched on the relationship between the vascular bundle of stem and flower or between that of foliage and scaly leaf.

Maekawa and his collaborators (4, 5, 6) have frequently pointed out the manysidedness in the leaf-stem relationship of vascular plants, from the view point of leaf-class conception. Depending on this conception, the writer investigated on the vascular course of Piperales.

Materials and Methods

Chloranthus japonicus collected at Arakawa-banks of Shiki and in Yono, Saitama Prefecture was used on occasion from the middle of March to October, being cultivated in a garden. The seedling, however, was managed in a frame. *C. serratus* was collected in Yono, and in Shiroyama, Kitatama District, Tokyo, and also near Kirifuri Fall in Nikko. *C. spicatus* and *Sarcandra glabra* have been cultivated in the frame of Koishikawa Botanical Gardens of the University of Tokyo or Imperial Gardens of Shinjuku and also the latter in garden near Yugawara, Kanagawa Prefecture.

After the fixation in formaline-acetic alcohol (formalin 2%, acetic acid 6% and alcohol 70%), they were taken down to paraffin through buthyl alcohol and then cut in section at 8 to 16 μ thick. The sections were stained with double staining, Heidenhein's haematoxylin and safranin. For understanding the dynamic structure of the bundles, other techniques such as a modified cleaning method with alkali treatment of Kasapligil (7) were also used.

Observations

1. External morphology

Chloranthus japonicus

Stem—It spreads subterraneously to form several stumps as subterranean stem, each nodes of which have lateral roots. The axillary bud to scaly leaf on the subterranean stem grows up as the terrestrial stem in early spring, with a terminal inflorescence. The terrestrial stem dies by frost, but several nodes of the subterranean stem with the axillary bud which is produced from September to October remain under the ground. This subterranean stem grows in the type of the sympodial branching (Fig. 1-4). The terrestrial stem grows up above the ground, and flowers on the top at the end of the same month. It is 5 to 8 cm high in flower, but reaches about 20 cm high in fruit.

Leaf—The scaly leaf is triangular, rather succulent, and 2 to 4 mm long, arranging in decussate phyllotaxis and with no appendages. On its uppermost part of stem, two pairs of the foliage leaves arrange in the same phyllotaxis just as the scaly leaves, and are 3 to 5 cm long, with a petiole and a pair of nail-like stipules at its base. The four leaves of the two pairs look like those in verticillate phyllotaxis, because the internode of foliage leaves is very short (Fig. 1-1). In flowering time the stems and the leaves are tinged with purple-brown and afterward each leaf grows and becomes green.

Inflorescence and flower—As the continuation of the arrangement of the foliage leaves, the inflorescence with 5 to 7 pairs of flowers reaches 2 to 3 cm long. The flower has a small triangular bract and no perianth (Fig. 1-2, 3). The single stamen is inserted in the abaxial side of the base of the ovary and trifurcates in slender lobes, two lateral ones of which have one theca, half of an anther, in each outer side of their base. The stamen is prominently white in flower. The pistil is one and has an undivided sessile stigma. The lower part of the globular ovary is wrapped up in a bract. It has a chamber with a pendulous and orthotropous ovule in the upper part of its inner abaxial side (Fig. 1-3, 4).

Chloranthus serratus

Stem—Both the terrestrial and subterranean parts are two or three times as long as *C. japonicus*, but are almost similar to the latter in the form. The seasons when the stem grows up above the ground and the flowers open are a month later than *C. japonicus*.

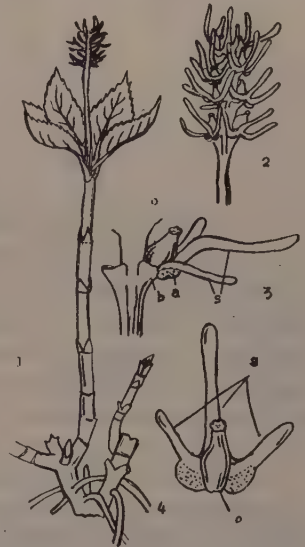


Fig. 1. *Chloranthus japonicus*. 1, entire plant. 2, inflorescence. 3, side view. 4, posterior view. o-ovary, s-stamen, a-anther, b-bract

Leaf—Scaly leaves are more than *C. japonicus*, by 4 to 6 pairs, but phyllotaxis is the same. Foliage leaves are 2 to 3 (or 4) pairs with the internodes shorter than those in the part of scaly leaves. This species, however, differs from *C. japonicus*, in that the internodes are not reduced to take a verticillate appearance to leaves (Fig. 2-1).

Inflorescence and flower—The very short part of terminal stem above the foliage part, has small opposite scaly leaves and thrives into two straight inflorescences. Between these two inflorescences, the remnant of central axis of stem remains, and develops often into a subsequent inflorescence or rarely further into 3 to 5 ones by its luxuriant branching. The plant with many foliage leaves shows constantly such tendency. The inflorescence is 3 to 4 cm long and like *C. japonicus*. The flower is wrapped up in a bract at the base. The form of pistil is like *C. japonicus*, but the trifurcated stamen is not, having two thecas of an anther on the inner side of the center lobe besides with each one theca on the two lateral ones. The yellow anther extends to the outside of ovary (Fig. 2-2, 3).

The ovule is rudimentary, and after the flowering, the inflorescence falls as a whole, without fruiting, by the supplementary growth of an absciss layer at its base. At about the same time, most of the axillary buds of both scaly and foliage leaves begin to grow, sending forth, at every node, two or three inflorescences with one to two pairs of the poor foliage leaves. The pistil of these inflorescences does not differ from that of the flower on the terminal one but the stamen is abortive and especially reduced in number of the anther. Their pollens are a few and most of them are withered and not stainable by acetocarmin. The rate of germination of the pollen and the mechanism of fertilization have not been researched. Notwithstanding these features in flowers, the axillary inflorescences come into often fruit-bearing.

Chloranthus spicatus

Stem—It is a dwarf evergreen shrub with green, rather slender but perennial stem composed of somewhat elongated internodes.

Leaf—There are only two or three pairs of the scaly leaves at the base of the shoot growing in spring. The foliage leaves arrange in opposite phyllotaxis, and are petiolated. They are somewhat coriaceous, oblong and about 3 to 4 cm long, and serrate on their margin. As they are like the

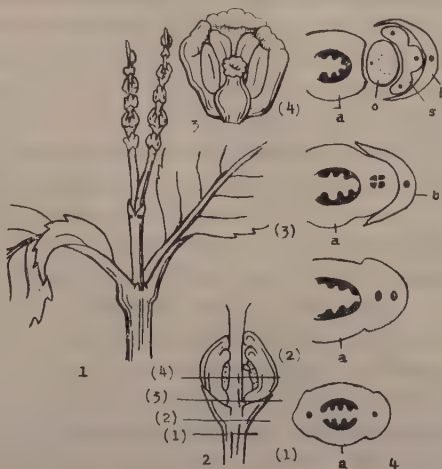


Fig. 2. *Chloranthus serratus*. 1, inflorescences. 2, one pair of flower. 3, single flower. 4, diagram of cross section at each level; corresponding with the numbers of 2, respectively. o-ovary, s-stamen, b-bract, a-axis of inflorescence.

leaves of tea, they are called "Cha-ran," meaning "Dwarf fragrant plant with the tea-leaf."

Inflorescence and flower—The newly growing terminal stem has small triangular scaly leaves alone with no foliage ones. The inflorescences spread from the axils of these triangular scaly leaves, and usually make two or three stairs. The flower is like *C. serratus*, though somewhat smaller than the latter. The fruit hardly matures, falling off at the end of flower time, and leaves the bract. Even when rarely it matures, the author has not yet collected any seedling.

Sarcandra glabra

Stem—It is an evergreen shrub; its stems are erect in 1 m high, and remarkably swollen at the node.

Leaf—The leaves are opposite, somewhat coriaceous, elliptical and 5 to 10 cm long, with rough serrations on margin. They are provided with small triangular stipules at the base of the petiole.

Inflorescence and flower—

From late April to June the inflorescence successively elongates from the top of the short shoot developed in the axil of the scaly leaf. The flowers are arranged in decussate, wrapped up in a bract in its lower part, and have no perianth. In the pistil, there are an undivided stigma, a short style, and an one-chambered ovary with a pendulous-orthotropous ovule within its upper corner. Many remarkable resemblances can be found in these organs between this species and the others in *Chloranthus* above mentioned. The stamen, however, is entirely different from all of them in the

point that it has a thick rod-shaped body without trifurcation and that it is inserted to the abaxial wall of ovary, having two thecas of an anther on both sides (Fig. 3-1).

2. Vascular course. The pattern in which the vascular courses of foliage leaves are separating from the stem, is entirely similar to each other in three species of *Chloranthus* and *S. glabra*, and almost coincides with the data in the report on *S. glabra* by Swamy and Bailey (3). The present paper informs the observations on the relationship not referred by Swamy between the bundles in *C. serratus*, which are supplied in every parts of the stem, the bract and the flower. The differences from the other species are further pointed out.

The vascular course, particularly, the mode of relationship between the

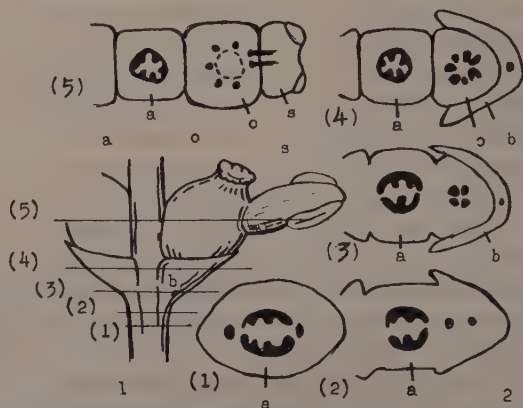


Fig. 3. *Sarcandra glabra*. 1, flower. 2, diagram of cross section at each level; corresponding with numbers of 1, respectively. o-ovary, s-stamen, b-bract, a-axis of inflorescence.

bundles running in each organ, can be observed by the continuous sections prepared from the materials of every stages, beginning with winter bud formation.

In the adult, by the gradual elongation of the internodes and by the remarkable enlargement of the cells in parenchyma, it is not easy to trace each bundles. Therefore, the branching course in early stage is principally observed. This is also due to that, the writer understands the early stage in development as the basic pattern.

Stem and nodal anatomy — The stele in the stem consists of main four-streaked meristeles and small ones in the same number arranging radially in the alternation of main ones. Each small bundle is divided into 2 to 3 streaks in the subterranean part of the stem, but is brought together in one streak upward in terrestrial part.

The vascular pattern of the scaly leaf is similar to that of the foliage leaf. Since the lamina does not develop in the scaly leaf, however, five bundles separated from the stele, run insides toward the end of scaly leaf. The small bundles (corresponding to Fig. 4-2-A) in the center of midrib occasionally do not enter into the scaly leaf and then fade away in the cortex of the stem. On the contrary, the small bundles (corresponding to B_1' , B_2' in Fig. 4-2) bifurcate and run into the scaly leaf. Not only the scaly leaf has the same running of bundles as the foliage leaf, but there are several deformities which indicate that one of a pair of foliage leaves coincides with the scaly leaf. Thus it is assumed that both leaves have originally the same primordial form.

The foliage leaves attach to the stem, in the median plane through the two sets of each two main meristeles. The small bundles (A_1 , A_2 in Fig. 4-2) between main meristeles are supplied in the cortex deviating outwards from the stele. In this level, the main meristeles are constricted and equally divided (C_1D_1 , C_2D_2 in Fig. 4-2). The small bundles B at right angle to A-A, get away from the stele simultaneously with A, but are divided immediately between the steles or in the cortex ($B_1'B_1''$, $B_2'B_2''$ in Fig. 4-2). A_1 and D_1D_2 are supplied to the petiole and become the main leaf-trace. Furthermore, B_1' and B_2' traverse in the cortex and become the subsequent leaf-traces, which run into both sides of $D_1-A_1-D_2$. So that, in the lamina, $D_1-A_1-D_2$ build up the midrib and $B_1'B_2'$, the veinlets which run inside the leaf-margin. The net veins of the blade are all diverged from D_1D_2 . A_1 , the middle member of $D_1-A_1-D_2$, fades out in midway of the lamina, and D_1 and D_2 fuse together in the tip.

After the separation of the leaf trace, the main bundles in the stele of the stem (C_1C_2) reduce by half, transfer towards the center of the stem, and separate simultaneously small bundles (F, G) from both ends. F and G form the small bundle by fusing each other soon after their separation. Thus, the first arrangement of the stele is recovered again.

When the leaf started from the stem, the stipules of foliage leaf, as a merely parenchymatous tissue without bundle, remain in the basal sides of the leaf. In the above-mentioned stage, the meristems develop between the

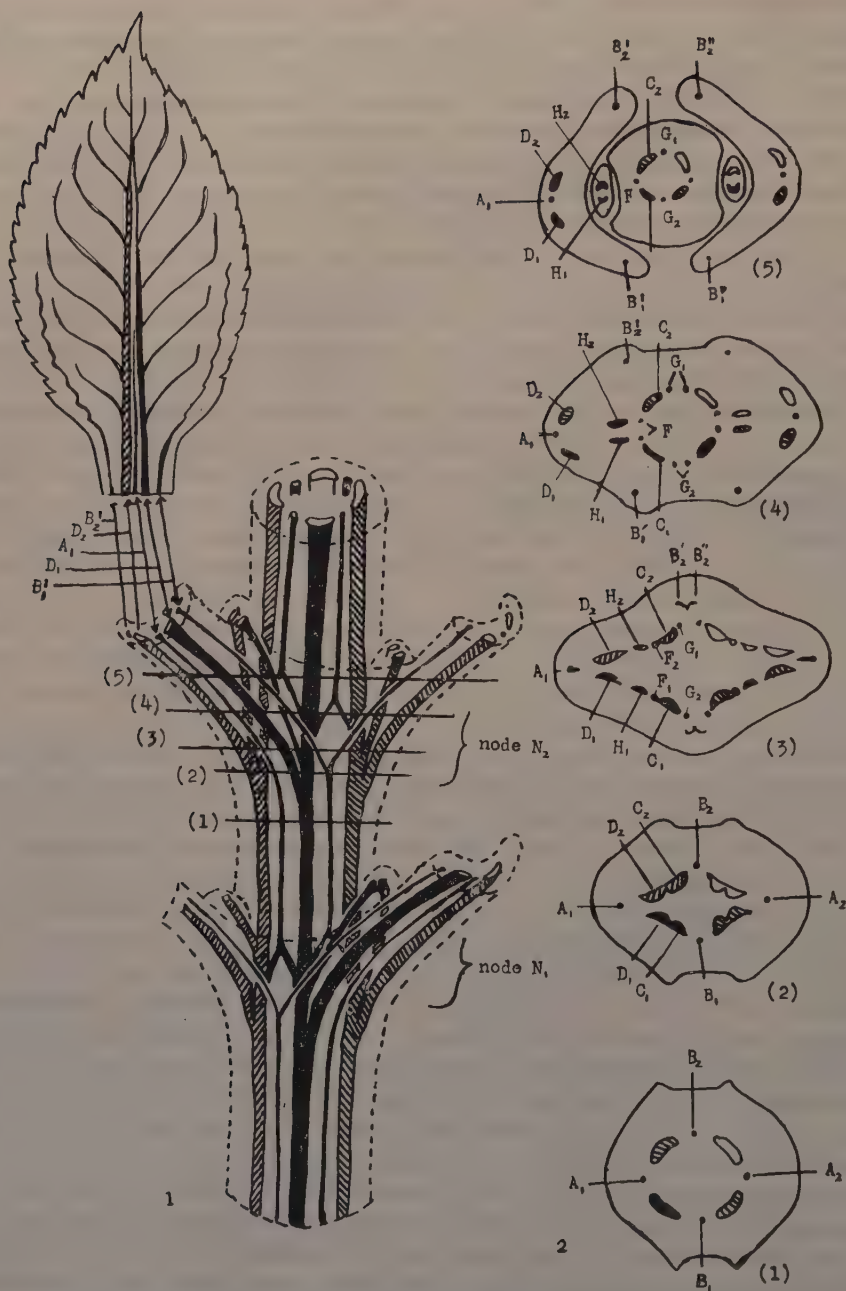


Fig. 4. Vascular courses of *Chloranthus serratus*. 1, diagram of the relationship of stem and leaf. 2, diagram of cross section.

leaf and the stem to form the axillary bud, the small bundles (H) between C and D are supplied to the axillary bud. These bundles in the axillary bud, which are seen as lunar shape in cross section and are two in opposite arrangement at first, develop in the stele with four main bundles, when the central part of the lunar portion runs away as the trace of prophyll.

Inflorescence and flower—After a few pairs of foliage leaves are made up through several bifurcations of bundles as above, the stele can not entirely recover its arrangement. The small bundles are arranged in a loop separating from each other, and nearly form a ring. Thus the bundles of scaly leaves at the base of inflorescence do not so regularly bifurcate as in the case of the foliage leaf. After the small bundles run away altogether towards the peripheral portion from a part of the "ring," two scaly leaves in opposite are made up, separating from the stem, in somewhat tubular form. The bundles supplied in the scaly leaves hardly have the leaf-gap.

The bundle supplied in the flower is one, and is separated from the stele of the axis of inflorescence by a small gap (Fig. 24). The first division of this bundle, immediately occurs in "periclinal." The divided bundle in the outside is supplied to the bract, and the inside one makes up four bundles in the place just above the separation of the bract bundle, with the simultaneous divisions both anticlinal and periclinal. The three bundles of them become those which are supplied in each branches of trifurcated stamen, and the one ascends through the adaxial side of ovary. While in the upper part of the flower, the bundle of ovary and that of the middle lobe of the stamen are just in the same plane with the bundle of the bract. Therefore, the arrangement of these four bundles in the flower, is twisted from the first axis of division. After the bundle is supplied to the ovary, it undergoes bi- or trifurcation at the chalazal side of the ovule, one branch runs into the ovule and, the others the stigma. In the small flower of *C. japonicus* or in the rudimentary flower of the axillary bud in *C. serratus*, the bundle supplied in the style is not found frequently. All the bundles in flower are merely an aggregation of two or three tracheids.

The running patterns of the bundles in leaves are similar each other in four species, *C. japonicus*, *C. serratus*, *C. spicatus* and *S. glabra*. Those in inflorescences are similar in three species of *Chloranthus*, but in *Sarcandra* four bundles, after separating one which is supplied to the bract, further bifurcate to arrange in circle in the wall of ovary. By the reserved bifurcations occurred locally in some cases, there are generally 5 to 8 bundles.

Discussion

Through these observations it is obvious that there are remarkable dichotomies in the floral part of *Chloranthus*. A particular attention must be paid to the fact, that these are similar to the vascular course of the female inflorescence in *Ginkgo biloba*.

In *G. biloba*, the bundle of the leaf, having the inflorescence in the leaf-

axil, separates from the stele of the short branch. The division occurs on dorsiventral plane at first and then in transversal plane. The two bundles in the outside form the leaf trace of the foliage leaf, while the inside two become four bundles with a further division in the same direction, and then are supplied to the "pedicel."

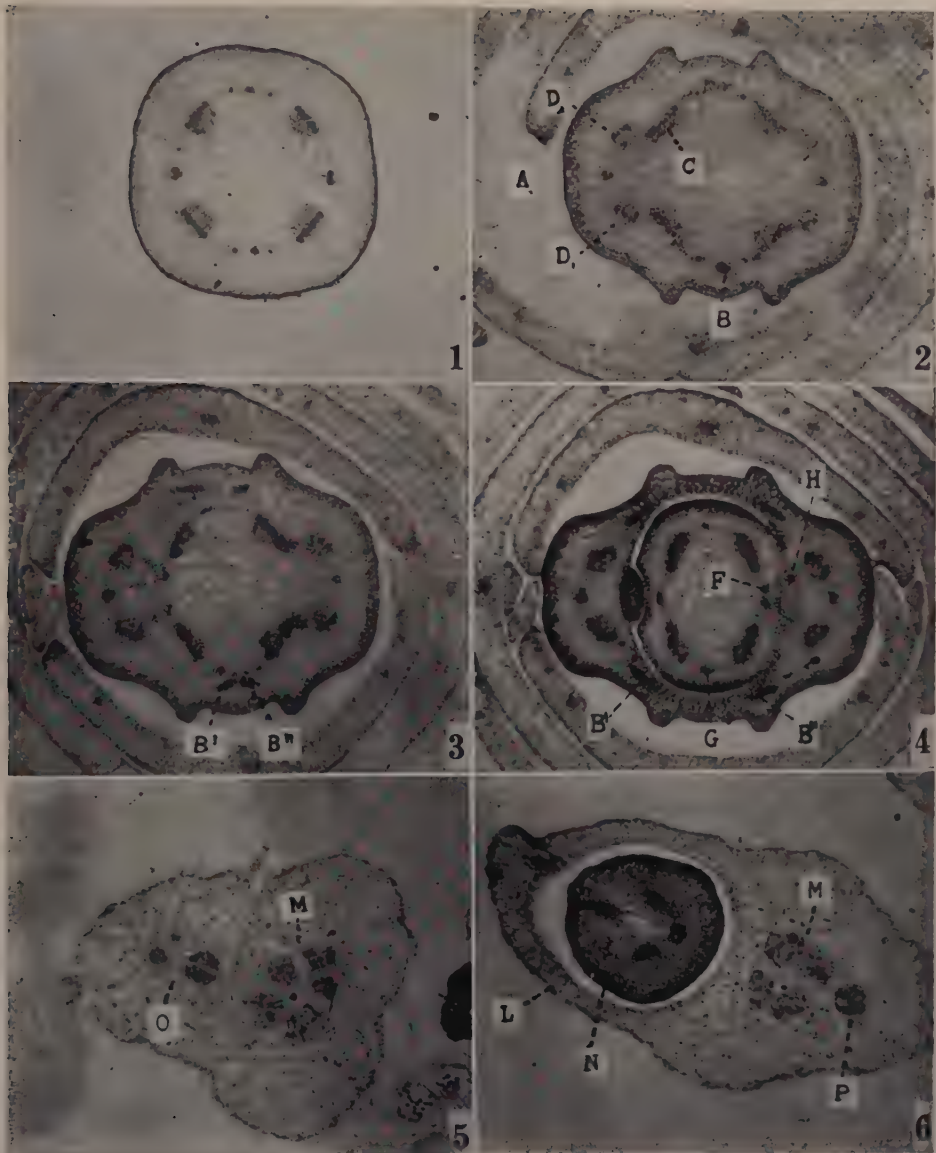
Chloranthus is different from *G. biloba* in only one point that the bract and the flower are separated by the first division. The bract in *Chloranthus* corresponds to the foliage leaf in *G. biloba*, and the four bundles are supplied to the floral part in the former, and to the "pedicel" in the latter. There is also similar relationship, too, between *Chloranthus* and *Ophioglossum* of Pteridophyta. The bract in the former corresponds to the sterile frond in the latter and the flower, to the fertile frond. The flower carries a macro-sporophyll-lobe with three micro-sporophyll-lobes. Although the vascular course in *Sarcandra* is considerably complex, it can be reduced to this type.

The fact, that the vascular bundles in the midrib of the leaf are two, is found in a few examples in Dicotyledoneae. In the somewhat undeveloped leaf of *Chloranthus*, the small bundle in the midrib fades away in the cortex of the node, and the leaf is formed by only two main bundles. In the small axillary branch which has no small bundle in the stem, the leaf is formed only by the vascular course of the main meristele, too. According to the study of Swamy, the fact, that *Ascarina* in Chloranthaceae belongs to this type of the vascular course, seems to suggest the close relationship between those two genera. Swamy pointed out that the leaf of *Hediosmum* has five bundles in the midrib. This fact demonstrates that two main bundles of *Chloranthus* or *Ascarina* repeat once more dichotomies.

The observations of the branching of the small bundles need keen attentions. The small bundle either in the seedling or in the axillary bud appears ontogenically later than the main bundle. Although several veins appear irregularly at first, they fuse together independently of the thickness of the stem and thus gain the regular bifurcation in the upper part of the stem. When the leaf is formed, the small bundle separates from the main meristele prior one node to the division in that main bundle (Fig. 4-1). For example, in this figure, the small bundle separates from the node N_1 , but the large one from the node N_2 , and then they run together into the leaf of the node N_2 .

The writer has extended her observations to other families in Piperales. Having already obtained some interesting results that the vascular bundles in the flowers of *Houttuynia cordata* (Saururaceae) and *Piper Futokadzura* (Piperaceae) are similar to those of *Chloranthus*, it seems confirmative to the writer that the applicability of *Chloranthus*-pattern will be found generally in Piperales.

Here I wish to express my hearty thanks to Dr. Fumio Maekawa in Botanical Institute, Faculty of Science, University of Tokyo, for his kind guidance and encouragement in the course of the work. Thanks are also due to Mr. Masayuki Takeuchi for his helpful support in the photography.



Stem and flowers of *Chloranthus serratus*

1. Cross section of the stem.

2, 3, 4. Cross section of the node of foliage leaf. A: Small bundle of the midrib. B: Small meristele of the stem. B', B'': Small bundle of the leaf margin (Small leaf trace). C: Main bundle of the midrib. (C main meristele in them).

5. Cross section of the inflorescence axis. M: Stele of axis. O: Bundles supplied to floral part; a small one to bract, and a large one (already bifurcated) to the flower.

6. Cross section of the base of ovary. L: Bract. M: Inflorescence axis. N: Base of ovary (showing four bundles, adaxial one, supplied to the ovary, and others to stamen). P: Branch trace of the next flower.

Summary

1. Under the conception of the leaf-class, this report deals with the vascular course of *Chloranthus japonicus*, *C. serratus*, *C. spicatus* and *Sarcandra glabra*.

2. The vascular courses in the vegetative parts of these four species are similar to each other. The results of the observations nearly coincide with Swamy's reports, in respect to the vascular course of the foliage leaves in *Sarcandra* and *Chloranthus*. But it quite differs from them in its nodal anatomy which pointed out that the small bundle separates from main meristele, already in the node prior by one internode to the point of the leaf insertion and that it runs parallel with main meristeles to the next node.

3. The vascular course in the floral part is similar to each other among these three species of *Chloranthus*, but is slightly modified from them in *S. glabra*. Furthermore, they are also similar to the vascular course of the female inflorescence in the short branch of *Ginkgo biloba*. According to these facts, it is considered that the floral parts of these species belong to the G-type leaf-class.

4. The division of the bundles of foliage leaf is also due to dichotomy.

5. The characteristic branching in the small bundles of the steles is interested topologically in the phylogenetic relationships to other genera.

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Anatomical and Morphological Studies of Japanese Species of the Ophioglossaceae.

II. Rhizome and Root*

By

Yoshitomo NOZU

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In the previous paper (1955) the writer described in detail the vascular system in the phyllomophore of 15 Japanese species of *Botrychium*, *Helminthostachys* and *Ophioglossum*, and reached to the conclusion that this organ, which has been regarded as the base of the petiole or a part of leaf by the former authors, should be considered as an intermediate organ between the rhizome and the petiole, or a special type of mesome.

In the present paper, the writer deals with the morphological and anatomical features in the rhizome and roots of Japanese species, because, notwithstanding numerous papers on the rhizome and root of the Ophioglossaceae have been published during the last hundred years, our present knowledges on the morphology and taxonomy of this interesting group have remained rather vague.

1. Observation

A. Rhizome. It is well known by the previous studies of various authors, that the stelar system of the rhizomes of the Ophioglossaceae is characterized by ectophloic solenostele or dictyostele enclosed only by an external endodermis, and the presence of secondary tissues in a few species.

In subgenus *Sceptridium* of *Botrychium* the rhizome is generally short and upright, though, in *B. ternatum* it reaches sometimes more than 30 cm in length. Also, in *B. japonicum* the rhizome can be roughly divided into the three conditions; (1) the rhizome is short and upright, and provided with long, rather closely arranged roots; (2) the relatively long slender rhizome runs almost horizontally, which shows a dorsiventral character, roots being arranged on its ventral side; (3) an intermediate condition between (1) and (2), and the rhizome is fairly short and runs obliquely, the condition of the roots resembling (1). Distinction of 3 conditions is, however, frequently obscure according to the ecological conditions, though (1) is more frequent than (2), while (3) is quite rare.

The internal structure of the rhizome in most species of this subgenus is approximately identical with each other, except in their size. Here,

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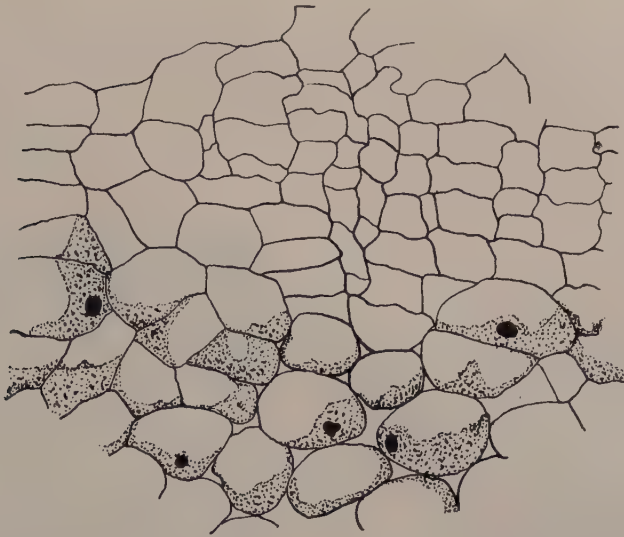


Fig. 1. *Botrychium japonicum*. Part of cross section of rhizome, showing cork layer. $\times 150$.

B. japonicum is chosen as a typical example. The rhizome is 4-8 mm in diameter and the structure is rather simple. The epidermis, in most cases, consists of a layer of cells which are relatively large and provided with thick outer walls, but they are eventually collapsed by development of a cork layer. It begins immediately under the epidermis and develops centripetally (Fig. 1). This cork layer is usually composed of 6-8 cells, but no trace of cork cambium is observable. Under the cork layer, there is a thick fundamental tissue of parenchymatous cells. The whole of the cortical tissue contains large quantities of starch grains. The outer region consists of large round cells, 10 or more layers in thickness, while the inner region, of about 25-30 layers of smaller round cells with intercellular spaces. Within the inner cortex, is situated the solenostele whose one side is concave. The metaxylem is composed of tracheids arranged in more or less regular radial rows, row of tracheids deviating from 7 to 10 in number. The elongated pits on their walls are observable even in the cross section as is shown in Fig. 2. The thick ring of tracheids is

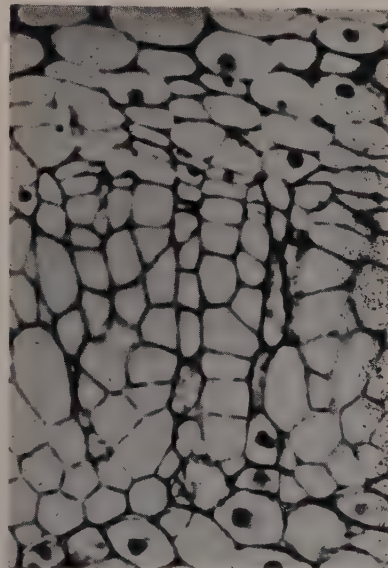


Fig. 2. *Botrychium japonicum*. Part of cross section of rhizome, showing tracheids and a single row of parenchymatous ray cells. $\times 1000$.

interrupted at intervals by a single row of parenchymatous ray cells. Some rays run through the thick ring of tracheids and others stop on the way. The protoxylem lies in the inner face of the xylem mass. Outside the zone of tracheids there can be seen a layer of the cambium. Outside the cambium lies the zone of sieve elements, whose walls are fairly thickened, but do not stain strongly with safranin. The endodermis is very conspicuous, as the Caspary strips on the lateral and inner walls stain very strongly with safranin. The pith usually consists of homogeneous and thin-walled parenchyma of relatively large cells. The same condition is found in *B. nipponicum*, *B. robustum* and *B. ternatum*. There is, however, a fact worth noticing that the isolated tracheids are rarely found near the periphery the pith of the rhizome in *B. japonicum* (Fig. 3).

The leaf trace commonly departs from one side of the hollow cylinder of the underground rhizome, leaving a distinct gap. When a leaf trace departs, at first a part of collateral endarch bundle with a single protoxylem group somewhat projects on one side of the stele. Soon the parenchyma cells of the pith intrude to both sides of the sector of tracheidal tissue which moves gradually outwards without cutting the phloem and the endodermis. Finally, the trace is completely separated from the stele. Thus, the trace leaves the stele as a somewhat curved bundle, group of tracheids being mostly arranged in radial rows, as is found in the stele.

In *Eubotrychium*, the rhizome is generally smaller than that of *Sceptridium* in diameter, but the structure is quite similar with each other. For example, it is about 1.5 mm in diameter in *B. lanceolatum*. The epidermis shows a normal type and its free face is thick and suberized, but it is collapsed. Below the epidermis lies the cork layers, which are smaller than those of *Sceptridium*, but their walls are thin like those of *Sceptridium*. The cortex is rather narrow, about 10-15 cells in layers, consisting of relatively angular cells on the outside and roundish cells on the inner side. The solenostele consists of the irregular ring of 3-4 tracheary tissue with no clearly developed medullary rays, and of the phloem of 3 cells in layers. The outer portion of the phloem is composed of a layer of thick-walled cells. The endodermis cells are relatively clear, and some of them show

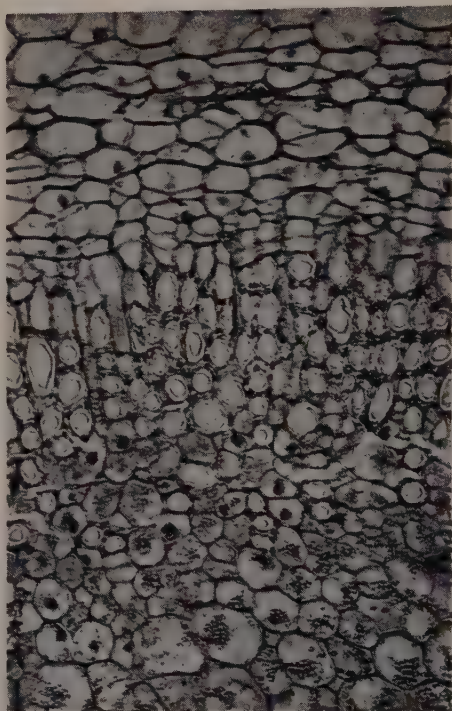


Fig. 3. *Botrychium japonicum*. Part of cross section of rhizome, showing isolated tracheids in the pith. $\times 320$.

the radial thickening of the walls. The cambium is absent and apparently there is no secondary increase (Pl. III, A), but we rarely find the cambium in large specimen of *B. Lunaria*.

In this subgenus, manner of the departure of the leaf trace is the same as in the case of *Sceptridium*.

In the subgenus *Osmundopteris* the rhizome is larger and stronger than the foregoing 2 subgenera. Here, *B. structum* is chosen as a typical example. The rhizome is usually 4.5-8, rarely more than 10 mm in diameter. The epidermis is characterized by a very thick outer wall and is eventually collapsed by the development of the cork layers. The cortex is consisted of about 45 layers of thin-walled cells. One of the characteristic features in the rhizome lies in the stele, that is, the xylem is composed of a thick ring of radially arranged, 20 or more tracheary tissue which is interrupted at intervals by uniseriate rays. The xylem is endarch. The activity of the cambium of this subgenus is more vigorous than in the case of *Sceptridium*. The phloem is from 3 to 5 layered. The extreme outer portion of the phloem is composed of a layer of thick-walled cells. Just outside of the phloem, there lies an endodermis whose radial walls are thickened and lignified, and stained strongly with safranin.

In this subgenus, manner of the departure of the leaf trace is roughly same as in the case of *Sceptridium*, but the curvature may be stronger than those of *Sceptridium*.

In *Helminthostachys zeylanica* the rhizome is creeping and markedly dorsiventral, the characteristically branched leaves being arranged in 2 rows on its dorsal side. The rhizome is very thick, mostly 8-12 mm, rarely more, in diameter. The epidermis consists of a layer of rounded cells. The cortex is bulky and consists of about 50 layers of rounded parenchymatous cells with prominent intercellular spaces. The

outer wall of the outermost layer of the cortex is thickened, suberised, and brown in colour. All the cells of the cortex contain starch grains. The vascular region is distinguished by the endodermis (Fig. 4). The stele shows a solenostelic type, in which foliar gaps do not overlap as a rule. Differing from the other members of the Ophioglossaceae, the xylem is mesarch. Namely, the protoxylems, which are narrow and stain less deeply, are present at intervals in the tube of xylem. The inner and outer xylems, as Lang states, develop more or less radially both at the ventral and dorsal sides of

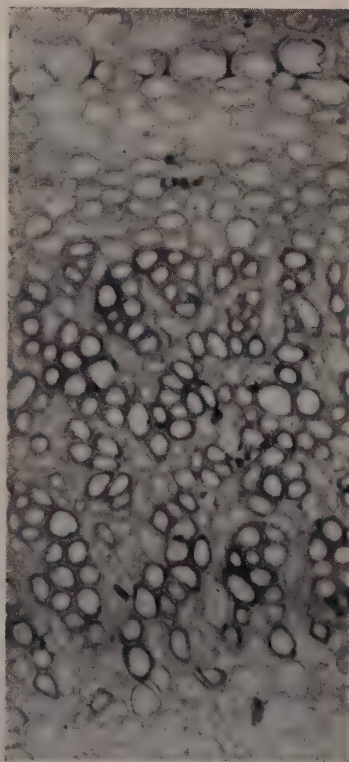


Fig. 4. *Helminthostachys zeylanica*. Part of a stele, showing mesarch protoxylem, $\times 150$.

the stele respectively. The metaxylem consists of tracheids with characteristic oval pits. In the longitudinal section, it is found that tracheids are very irregular in form as well as in size. The secondary thickening is entirely absent. Around the xylem core, a few layers of phloem cells can be seen. Pith is well developed and consists of the parenchyma of large cells. The internal endodermis and the phloem cannot be found at any places of the pith. The manner of departure of the leaf trace from the dorsal side of the rhizome is very complicated. It has been observed that the manner of the leaf trace has 2 cases. One case is as follows: The trace projects outward as a rounded hump. Soon one side of it separates from the vascular ring, but endodermis remains still continuously. Subsequently the trace separates completely from the stele and takes the form of a C. Soon after it closes into the form of a O, and it is further transformed into a clepsydroid type (see part I. Pl. 2. E). When the trace approaches the outer part of the cortex, it is divided into two.

The other is as follows (Fig. 5). Firstly, the outer contour of the xylem at the upper both sides in xylem ring projects as a rounded hump. Soon parenchymatous cells in the xylem ring aggregate internally to the xylem. The outer xylem projects still more, and then takes the form of Clepsydroid type, enclosing parenchymatous island within complete 2 rings of xylem. As a rule, one side of the rounded hump gradually is shut off from the xylem ring by the intruding parenchymatous cells.

The trace continues to move outwards to be cut off also from the xylem ring at the opposite side. Thus the trace becomes completely abstracted from the stele, and the endodermis and phloem still continue to those of the stele. About the middle part of cortex, the endodermis and phloem completely enclose the separated leaf trace, and still continue to move as they are so far as outer part of the cortex.

In subgenus *Euophioglossum* the rhizome is generally short and upright, and is smaller than those of the other genera of the Ophioglossaceae. Externally, the root develops much more than the rhizome. Here, *Ophioglossum littorale* is chosen as a typical example, the rhizome is usually 1.0-3.5 mm in diameter. The epidermis consists of a layer of cells whose outer wall is very thick. The cortex consists of about 30 layers of thin-walled cells. The stele is solenostelic, but more often there is an overlapping of gaps showing a dictyostelic condition (Pl. III, B). The xylem portion of a meristele is endarch in arrangement. The metaxylem is composed of irre-

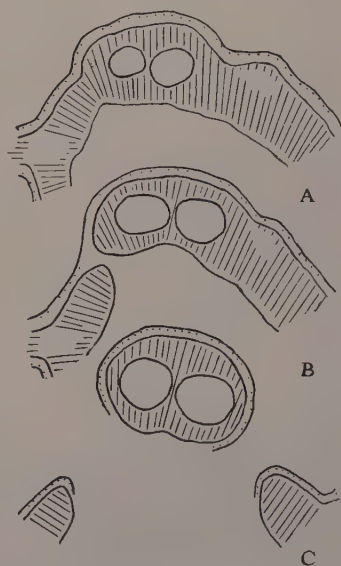


Fig. 5. *Helminthostachys zeylanica*. Diagrams of a series of sections through the leaf trace in course of branching. $\times 25$.

gular ring of 4-5 rows of tracheary tissue with no clearly developed secondary thickening. The phloem is a layer of 4 or 5 cells in thickness. The endodermis cells are relatively clear and its lateral walls are thick. The pith is composed of a homogeneous, thin-walled, relatively large parenchymatous cells, containing a number of starch grains.

In this subgenus, the manner of departure of the leaf trace is the same as in the case of *Eubotrychium*. The single trace arises from the solenostele or dictyostele of the rhizome as a slightly curved bundle. The trace remains undivided as it rises through the cortex, but in large species the trace may show further divisions before it reaches the base of phyllomophore.

In subgenus *Ophioderma*, e.g. *O. pendulum* the rhizome is larger than that of *Euophioglossum*, mostly 5-10 mm in diameter. The cortex composed of uniformly thin-walled parenchymatous cells. The stele of the rhizome is of a kind of perforated dictyostele. It consists, in cross section, of some meristeles arranged in a circle, which are differentiated into large and small. The meristele consists of ectophloic bundle, and the xylem consists of a mass of tracheids of from 3 to 5 layers. The phloem is composed of a single layer of the cell, and there can not be recognized a clear endodermis in its outer side. The pith is in all respects similar to the cortex. From a gap 3 or 4 traces originate and they run within the cortex, and each is divided into from 6 to 12 in total before enter into the base of the phyllomophore.

B. Root. The structure of the roots has been also studied by many previous authors, and it is well known that they show normal root characters, the cortex consisting merely of parenchymatous cells, and in some species containing mycorrhiza.

In the subgenus *Sceptridium* the root is fleshy and long, about 2.5-4.5 mm in diameter. The roots freely branch monopodially. The root tip is provided with a tetrahedral apical cell which is covered by a root cap (Fig. 6). The epidermis cells are relatively large and their free face is thick and suberized. The cortex is wide and constitutes of about 25 layers of parenchyma cells, which contains starch grains. Sometimes, the cork layer is formed in the cortex of the old root, and it is roughly same as the case of the rhizome.

The stele is usually triarch or tetrarch (Pl. IX, A), rarely polyarch, whose xylem groups are separated by parenchymatous cells (Pl. XI, C) and surrounded by a very distinct endodermis. The phloem elements lies very clearly. An example of

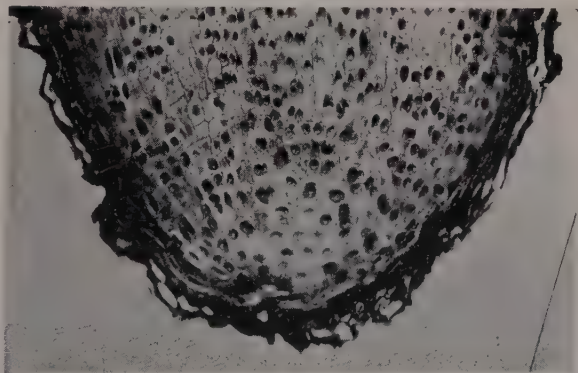


Fig. 6. *Botrychium japonicum*. Longitudinal section of root tip, showing apical cell. $\times 80$.

branching of the tetrarch root is shown in *B. japonicum*. When the root itself branches, one of xylem groups becomes more massive and then separates from the main root as one rootlet. When the root has 2 rootlets, 2 groups of xylems become more massive and then separate as 2 rootlets. The stele of the rootlet is monarch at first.

In the subgenus *Eubotrychium* the root is provided with a tetrahedral apical cell as in the former case. It is about 0.5 mm in diameter and the stele is diarch, or rarely triarch. The epidermis cells are approximately equal in size and their outer walls are somewhat thickened. The cortex is of 8-12 layers containing starch grains as seen in *Sceptridium*.

In the subgenus *Osmundopteris*, the root has a tetrahedral apical cell as in the case of other subgenera of *Botrychium*, but it shows a more regular segmentation. This root generally resembles to that of *Sceptridium*; that is, the innermost layers of the cortex consist of a few layers of smaller cells and the stele is frequently tetrarch, or rarely polyarch. An example of branching of the hexarch root can be shown in *B. virginianum*, in which 6 xylem groups, large and small, are arranged alternately. When the root branches, a large xylem



Fig. 7. *Ophioglossum littorale*. Longitudinal section of root tip, showing apical cell. $\times 55$.

group becomes more massive and then separates so as to form the rootlet.

In the genus *Helminthostachys* the root branches monopodially and is usually larger than the case of the rest of the Ophioglossaceae.

The apical cell is also tetrahedral. The bulky cortex consists of round parenchymatous cells with prominent intercellular spaces among them. Their cortical cells contain large quantity of starch grains. The stele is from hexarch to heptarch (Pl. IX, E).

Manner of the branching of the root is not very different from that in *Botrychium*.

In the subgenus *Euophioglossum*, the root is rather slender, hairless and it does not branch. The apical cell is tetrahedral and the development of the root cap is rather poor (Fig. 7).

The free faces of the epidermal cells are thickened and suberized. The massive cortex is consisted of about 10 layers of simple parenchymatous cells and mycorrhizal zone is very conspicuous. The endodermis is so obscure that it is not easily determined. Abundant starch grains fill the cells of inner cortex. The stele is monarch (Fig. 8), which is occupied by a large solid mass of xylem, separated from the endodermis by a single layer of pericycle cells. The single mass of phloem lying opposite to the xylem is not much more than half as large.

In the subgenus *Ophioderma* the root is also provided with a tetrahedral apical cell and the root cap is not so well developed. The stele is from triarch to tetrarch.

C. Development of the rhizome and root in the young plants in some species of *Botrychium* and *Ophioglossum*. An earlier stage of the young sporophyte with the prothallus was already described in the previous paper (1954), but no details have been described

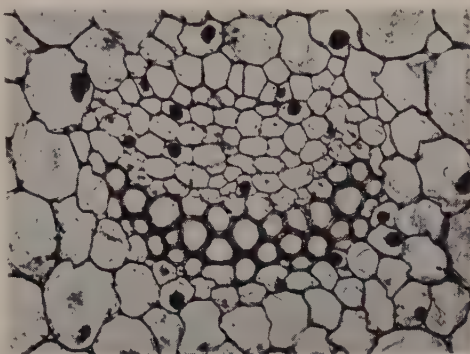


Fig. 8. Cross section of a root of *Ophioglossum littorale*. $\times 1000$.



Fig. 9. *Botrychium japonicum*. Diagram of longitudinal section of young sporophyte. $\times 25$.

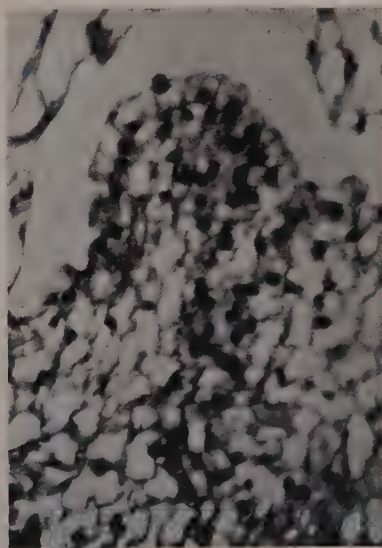


Fig. 10. Young sporophyte of *Botrychium japonicum*, in longitudinal section, showing apical cell of rhizome. $\times 1000$.

on the rhizome apex. A longitudinal section of a young sporophyte is shown in Fig. 9. The vascular bundle of the first leaf is plainly visible. The apical meristem of the second leaf is situated at its top and the procambium of the leaf trace is not yet developed (Fig. 10). The third leaf is found as a protuberance on the right hand of the second leaf, and at the base of the third leaf a conspicuous apical cell of the rhizome may be seen. The apical meristem of the rhizome is very limited in extent, being composed of a tetrahedral apical cell and a few small and inconspicuous cells.



Fig. 11. Developmental series of young sporophyte in *Botrychium japonicum*. Left: sporophyte 3 cm in length. $\times 1.5$.

In the next stage of the development, when the sporophyte reaches 3 cm in length, the first root is still short (Fig. 11); the weak first leaf consists of a slender phyllophore and ternately divided lamina. Fig. 12 shows a longitudinal section of a young sporophyte of this stage. A single vascular strand runs continuously through the leaf and root, and has nothing to do with the tissue in the apical region of the rhizome. The constituents of the root bundle are same with those of the leaf, though the stele of leaf is not yet developed sufficiently. The tetrahedral apical cell is very distinct, while the root cap is not so prominent. The apical meristem of



Fig. 12. *Botrychium japonicum*. Diagram of longitudinal section of young sporophyte, later stage than the one shown in Fig. 9. $\times 15$.

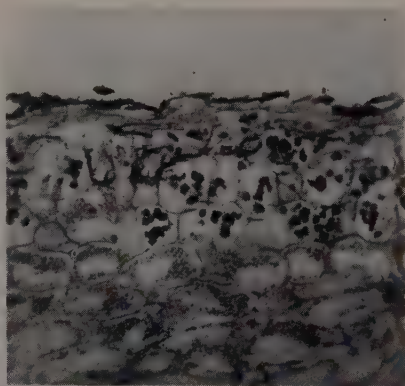


Fig. 13. *Botrychium japonicum*. Part of cross section of rhizome of young sporophyte, showing mycorrhizal fungi. $\times 200$.

the rhizome is well-defined. In this stage there occurs a cork cambium. Large parenchymatous cells inside of the cambium contain a large quantity of starch grains, and there can be seen the mycorrhizal fungi in the outer cells of the cortex (Fig. 13). The extreme outer portion of the vascular cylinder is composed of cells which are probably sieve elements whose walls is not stained strongly with safranin, though in the later stages of development these elements can be seen more clearly.

D. Adventitious buds on the root. In *Euophioglossum* adventitious buds are frequently found upon the roots. As a rule they are not terminal, but lateral in their position. A bud is usually formed on the upper side of a root, at point 2-8mm from the root tip. The initial of the bud, however, is formed near the apical cell of the root, though at first its differentiation is very difficult to distinguish, and it is somewhat later, that the initial cells of a bud may be recognized. Initial cells of a bud divide more rapidly than their neighbours and they form a mass of meristematic tissue situated in the inner cortex of the root in cross section.

In the later stage, some cells of meristematic tissue operate in the formations of the first leaf and the first root. The first leaf clearly occurs within the cavity formed schizogenously. During the bud is formed, the outer sheath is built up by raising of tissue of the epidermis and outer part of the root cortex. An incipient vascular strand traverses the inner cortex between the bud and the root stele. Finally, the bud bursts through the outer sheath. Thus the bud is endogenous in origin.

2. Discussion

The works on the rhizome and the root of the Ophioglossaceae have been published by many authors and it is clear from these studies that the rhizome and root are very variable in external features as well as in internal structures.

In the present paper, the writer wants to discuss on these organs of Japanese species of this family in order to compare with the results of researches of the others.

A. Rhizome. The rhizome of the genera *Botrychium* and *Ophioglossum* is in most cases short, upright and unbranched, while in the genus *Helminthostachys* it is long, massive and creeping with dorsiventral character. The rhizome of the subgenus *Ophioderma* is also dorsiventral. If the radial form of the rhizome is supposed to be primitive and the dorsiventral form to be derivative, *Botrychium* and *Ophioglossum* are more primitive than *Helminthostachys*, while *Ophioderma*, showing an epiphytical habit, can not be discussed together with these genera, which are characterized by subterranean rhizome.

Epidermis and cortex. The epidermis in the genus *Botrychium* eventually collapsed by development of a cork layer, while the free face of the epidermis in *Ophioglossum* is thickened and suberized, as stated by Holle (1875). The cork layers in *Botrychium* occur in the same way as observed by Baas-

Becking (1921) in *B. simplex* and *B. obliquum*, but he (1921) observed also the cork layer in a large pericycle surrounding the xylem of *B. simplex*. The presence of the cork layers may be due to the slow development of sporophyte. The cork layers can not be found in other living ferns, and it is of interest that in *Helminthostachys* this layer was not recognized.

Generally, the cortex in most species is thicker than that in higher ferns. Furthermore, it is significant that some of cortical cells contain mycorrhizal fungi. Farmer and Freeman (1899) found them in *Helminthostachys zeylanica* in its outer layers. Also, Campbell (1911) showed in his figures the mycorrhizal zone in the rhizome of *Ophioglossum moluccanum* and in the root of *O. pendulum*. In writer's materials the mycorrhizal zone is not always present, which probably may depend on the condition where the ferns are growing.

Xylem. In all the species of the subgenera *Sceptridium* and *Osmundopteris* the xylem of the rhizome is endarch and composed of secondary tracheids arranged more or less in regular radial rows. Endarch xylem is unusual form for ferns. In most species of the subgenus *Eubotrychium* and the genus *Ophioglossum* the xylem of the rhizome is chiefly composed of irregularly arranged reticulate tracheids, and in *Helminthostachys* the xylem consists of tracheids with characteristic pits and many parenchyma, the metaxylem is developed more or less radial at the center of the stele. In *Helminthostachys*, it is well known that the xylem is mesarch, and the writer's observation on inner xylem agrees with that of Lang, but according to him the degree of development of the inner xylem exhibits a wide range of variation. Lang remarked that the inner xylem is similar to the isolated tracheids in other species of the Ophioglossaceae, but the writer considers that the inner xylem differs markedly from the isolated tracheids.

The isolated tracheids are sometimes observed in the pith of *Botrychium japonicum*. According to Bower (1923) such isolated tracheids are frequently found in normal stems of *B. ternatum*, *B. virginianum* and *B. Lunaria*. Probably, their occurrence may be applicable to the interpretation of intrastelar origin of the pith.

The tracheids of the xylem of the rhizome exhibit spiral or scalariform marking on their walls as in most ferns. Wright (1920) found the torus of the bordered pits in the Ophioglossaceae, and Loughridge (1932) wrote in this connection that the tracheids of metaxylem and secondary xylem bears on their walls elongated or rounded pits, while Esau (1953) said that in Ophioglossales the helical and reticulate thickenings are combined with circular bordered pits of the type characteristic of the secondary tracheary elements of higher gymnosperms, and scalariformly pitted elements are completely omitted. However, in writer's observation these elements can not be recognized.

Phloem. The presence of the phloem restricted to the external face of the xylem shows also primitiveness of this family. Structurally, the phloem of the 3 genera are identical in essential points. It is a layer 4 or 5 cells in thickness. Russow (1872) pointed out the presence of the callus plates,

but on the writer's careful examinations it can not be seen. The phloem of *Ophioglossum pendulum*, a single cell in thickness, is separated from the xylem by 3 to 5 layers of parenchyma. This condition is one of the characteristic features of this species. The presence of internal phloem already observed by Farmer and Freeman (1899), Chrysler (1910), Lang (1915), and others, but in careful observation the writer unfortunately could not traced internal phloem and endodermis. It is evident that the appearance of internal phloem and endodermis indicates a step of the ontogenetic progression toward solenostele or dictyostele. Also, the presence of internal endodermis already observed by Poirault (1892) in *Ophioglossum Bergianum*, by Farmer and Freeman (1899) in *O. ellipticum*, by Lang (1915) in *Helminthostachys zeylanica*, and others. Lang (1915) described that the stele of *Helminthostachys* surrounded by the external and internal endodermis, is composed of 2 components in the xylem, i. e., inner and outer xylem. He also wrote about the progression from the juvenile to the adult type of rhizome, „When the full size was attained the stele showed the size and complexity of the full adult type, but had no internal endodermis.“ But in the writer's materials the internal endodermis is not recognized. It is probably related to the variations of the materials.

Pith. The pith in these 3 genera is usually consisted of a uniform, large and thin-walled parenchymatous cells containing a number of starch grains, as in the cortex cells. The pith is intra-stelar in origin, which may be justified from the ontogenetic facts observed in previous example, e. g., isolated tracheids of *B. japonicum*. The pith appears to owe its origin in all of relatively primitive ferns to an early change of destination of certain of the procambial cells.

Secondary thickening. As secondary thickening of the vascular tissue by means of a cambium is of a rare occurrence among pteridophytes, the presence of this tissue in the rhizome is an important characteristic of the Ophioglossaceae. Campbell (1911) said „In *Eubotrychium* the cambium is absent and apparently there is no secondary increase in diameter, but all of the large species of *Phyllotrychium* show a greater or less development of the cambium, which we have noted in the bundle of *B. virginianum*.“ The secondary thickening is found only in the subgenera *Eubotrychium*, *Sceptridium* and *Osmundopteris*. Lang (1913, 1915) observed in *B. Lunaria* the secondary thickening developed irregularly outside the primary xylem. In *H. zeylanica* it is characterized by the accessory xylem. As being clear in his figures, the regular development of primary xylem on radial rows seems to make the secondary thickening of xylem more easily. According to the writer's observation the secondary thickening in *Eubotrychium* is found only in large specimen of *B. Lunaria*. Lack of the secondary thickening in other species of *Eubotrychium* seems to be related to the weak development of aerial portion. The secondary thickening is seen only in pteridophytes of primitive type and we cannot find in other living ferns. This is an important character, because the occasional existence of secondary thickening in the part of the rhizome is considered to recall an ancient form.

Stele. Bower (1911) and Lang (1913) assumed that in *B. simplex* and *B. Lunaria* there is a cauline stele. In *B. japonicum* and *O. littorale*, however, it seems that there is no cauline stele as is shown in a reconstruction of stele (Fig. 14). This may undoubtedly be supported by Campbell (1911), West (1915), Baas-Becking (1921) and others with their many examples. There is no doubt that the solenostelic nature of the vascular system of the rhizome consists exclusively of leaf traces and the vascular supply of roots, as already mentioned in a previous paper (1955). Thus, the shoot of the Ophioglossaceae ferns generally consists of a conger of telomes and mesomes. Wilson (1953) remarked that „In the course of ontogeny each mesome was first a telome, being related to the mesome position as growth proceeded.“ If we accept his proposition, it may easily be considered that the rhizome originates the mesome in result and it will be interpreted that the rhizome is very similar with the phyllomophore essentially.

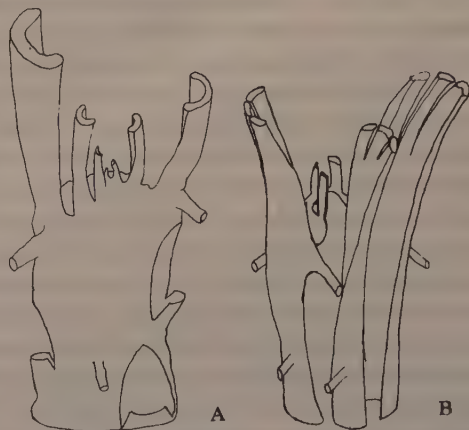


Fig. 14. Diagrammatic representation of the xylem near the apex of rhizome. $\times 8$. A, *Botrychium japonicum*; B, *Ophioglossum littorale*.

The structure of the stele, which is ectophloic solenostele with rather large gaps, is one of the characteristic features of the Ophioglossaceae. What does such an ectophloic state of the stele mean? Boodle (1901) and Gwynne-Vaughan (1903) mentioned that a solenostele was evolved from a protostele by a metamorphosis of the inner vascular elements into parenchyma. According to Jeffrey (1910, 1917), the parenchymatous tissue internal to the vascular ring is cortical in origin. Ogura (1938) said „Das Auftreten des gemischten Marks und der Bautypes der jungen Pflanzen lassen sich gut mit dieser Meinung in Einklang bringen. Schliesst man sich ihr an so Kann man homologe Erscheinungen hierzu bei den Osmundaceen und Coenopteridaceen finden. Diese Hypothese, dass der Ophioglossaceen-Typus eine Art der ektophloischen Solenostele sei die sich aus der markhaltigen Protostele ableite, deshalb nach ansicht der Verfassers die grossere Wahrscheinlichkeit in besitzen.“ The writer considers that the ectophloic stele of the Ophioglossaceous type is derived from the protostele from the view point of the ontogeny, and also inner endodermis or inner phloem is derived secondarily from parenchymatous cells of the pith. This similar type of stele is rarely found in *Anemia*, *Schizaea*, *Osmunda* and some species of *Gleichenia*.

In the genus *Helminthostachys* the stele is solenostelic and it consists of the xylem with scattered parenchyma. Though Campbell (1911, 1921) des-

cribed, in this connection, that the stele of the sporophyte in *Helminthostachys* was found in the same way as in *Botrychium*, the writer has observed that in the former the stelar structure was different markedly from that of the latter.

In conclusion, the stele of the rhizome of the family may be dividable into the following 3 types.

1. Solenostelic type

a) *Helminthostachys* type. The xylem is mesarch in origin and has scattered tracheids, provided with round or oval pits. The phloem is composed of a layer 4 cells in thickness. A single trace issues forth from the stele of the rhizome, and soon divides. This type is represented by *Helminthostachys zeylanica*.

b) *Botrychium* type. The xylem is endarch in origin and the tracheids, provided with scalariform pits, are arranged in radial rows of secondary tissues. A leaf trace departs from a single gap of the stele of the rhizome. The phloem consists of 3 or 4 cells in thickness. To this type belongs most species of *Botrychium* (*Sceptridium*, *Osmundopteris* and *B. Lunaria*).

2. Solenostelic or dictyostelic type

c) *Ophioglossum* type. The xylem is endarch and composed of irregularly shaped arranged tracheids, provided with scalariformly arranged narrow bordered pits. The phloem consists 4 or 5 cells in thickness. Most species of *Euophioglossum* and *Eubotrichium* belong to this type.

3. Perforated dictyostelic type

d) *Ophioderma* type. It consists, in cross section, of some meristemes arranged in a circle. The xylem is endarch in arrangement, which is composed of irregular shaped reticulate tracheids as in the former. The phloem consists of a single cell layer. From a leaf gap of the stele of the rhizome 3 or 4 trace issues forth. This type is represented by *Ophioglossum pendulum*.

These types form a rough series of reduction or specialization. This opinion is established chiefly on the basis of the following considerations.

1. It may be generally accepted that the mesarch condition is more primitive than the endarch one. Consequently the *Helminthostachys* type is more primitive than any other type in the family.

2. It is matter of importance that in some species of *Botrychium* there occurs the secondary vascular tissue by cambial activity which is not elsewhere among living ferns.

3. It must be considered also whether a single leaf trace arises or several ones from the stele of the rhizome. It is easily considered that in most cases of *Ophioglossum*, in which only one leaf trace departs from the rhizome, it is in a primitive monarch, as Bower (1923) assumed. It is further important that in the cortex of the rhizome the leaf trace breaks into several before it enters in the phyllo-mophore and the bundle course in the phyllo-mophore and leaf is determined by the number of the bundles thus divided.

Leaf trace. As has been clear in the description of the leaf trace in a previous paper, the Ophioglossaceae is dividable into following 2 groups.

- (1) One leaf trace; *Botrychium*, *Euophioglossum*, *Helminthostachys*.
- (2) More than 2 leaf traces; *Ophioderma*.

The first case, the leaf trace of *Botrychium* and *Euophioglossum* reaches to the base of the phyllomophore without increasing in number, while in *Helminthostachys* it is divided into 2 before enter into the base of the phyllomophore.

In the second case 2 or 3 leaf traces arise from the stele of the rhizome, and meanwhile, each trace increases in number and enters the base of the phyllomophore. This condition is rather usual in other Leptosporangiate ferns.

From the facts mentioned above, it may be said that the type of the leaf trace in the cortex of the rhizome provides the characteristics in each genus or subgenus. Especially in *Helminthostachys* and *Ophioderma*, manner of the departure of the leaf trace is far from the nature of *Botrychium* and *Ophioglossum*.

If single trace in the phyllomophore is regarded as primitive condition and the split the derived one, as Clausen (1954) described, mutual relationship in this group appears that *Sceptridium* shows the most primitive characteristic, *Ophioderma* the most advanced one, and *Eubotrychium*, *Osmundopteris*, *Helminthostachys* and *Euophioglossum* is intermediate between 2 subgenera *Sceptridium* and *Ophioderma*.

B. Root. The roots of the Ophioglossaceae are in most cases fleshly and devoid of root hairs. The roots of the subgenus *Euophioglossum* are characterized by possessing root-buds which serve usual method of propagation. Generally, roots of *Helminthostachys* are largest in size, these of *Botrychium* come to the next, while in *Ophioglossum* they are smaller. The thickened free faces of the epidermal cells are normal characteristic of the roots.

Presence of the concentric regions in cortex is also one of the characteristics of the roots. It is important that in the cortex of the Ophioglossaceae mycorrhizal fungi are present in the outer region composed of angular cells, though the mycorrhizal condition in the root is not so prominent as that in the gametophyte. As Eames (1936) mentioned, it is probably certain that the mycorrhizal habit in the root seems to be related to the development of the leaf.

Probably, the mycorrhizal fungi are infected in an earlier stage of the development of the root. It seems that this fungus is a phycomycete, probably *Stigeosporium* sp. On the other hand, cells in the inner region of the root cortex frequently contain starch grains.

The stele is always surrounded by the clear endodermis. The number of the groups of xylem of the root is not always constant, as Maheshwari (1934) and Ogura (1938) remarked. According to Boodle in *Botrychium ternatum* and *B. virginianum* the bundle is from diarch to triarch, but by further examinations more than triarch bundles occur as described above.

In the subgenus *Sceptridium*, as Campbell (1911) pointed out, the diarch

condition is probably secondary one. But, if we believe that the sporophyte had the vascular system of uniform structure, throughout the root, rhizome, phyllomophore and leaf, it is rather natural that the root in *Sceptridium* has diarch condition.

The subgenus *Eubotrychium* characterized by a relatively small sporophyte is always of diarch. In this respect there can be found well-defined line between *Eubotrychium* and *Sceptridium*.

In the subgenus *Osmundopteris* the roots are tetrarch, the number of which is more than those of *Sceptridium*. Internally, *Helminthostachys*, with their increased number of groups of xylem, appears to be the most specialized. In regard to this point, it is the most aberrant of the Ophioglossaceae, as Campbell pointed out. The roots of the subgenus *Ophioderma*, e. g., *O. pendulum* are diarch. But the number of bundles seems to be determined by size of the root, as Campbell (1911) and Petry (1914) remarked. In all the species of *Euophioglossum* the roots are monarch, as stated by Campbell (1911) and Bower (1926). It should be considered in this connection that we can repeatedly find the reduced form of the primary condition in other organs. However, Maheshwari (1934) found diarch or triarch roots in *O. fibrosum*. These structural facts may be conceived to be probable in unstable member of the ferns. These results are shown in Table 1.

Table 1.

Species	Branching of the root	Diameter of the root	Number of xylem in a bundle
		(mm)	
<i>Botrychium japonicum</i>	monopodial	2.5-4.5	3-4 (6)
<i>B. ternatum</i>	"	2.0-3.5	3
<i>B. nipponicum</i>	"	2.6-4.7	3-5
<i>B. Lunaria</i>	"	0.4-0.9	2 (3)
<i>B. lanceolatum</i>	"	0.3-0.8	2
<i>B. virginianum</i>	"	2.6-5.1	4-5 (6)
<i>B. strictum</i>	"	2.6-5.3	"
<i>Helminthostachys zeylanica</i>	"	2.6-4.7	7-10
<i>Ophioglossum pendulum</i>	"	4.5-10.0	3-4
<i>O. vulgatum</i>	unbranching	0.8-2.1	1
<i>O. reticulatum</i>	"	0.8-2.3	"
<i>O. littorale</i>	"	0.9-2.0	"
<i>O. ellipticum</i>	"	0.6-1.2	"

Notwithstanding the adventitious buds occur on the roots of most species in *Euophioglossum*, as Prantl observed, little has been known on their nature. Rostowzew (1891) attributed the inception of buds to a recent segment of the apical cell. Wardlaw (1929) put some general observations on Ophioglossaceae and recently (1953) he remarked that in an experimental investi-

gation of bud formation in *Ophioglossum vulgatum* the root buds are induced in both entire and decapitated isolated roots.

The bud formation is endogenous in origin which is in marked contrast to other ferns, as pointed out by Wardlaw (1953). In the present observation, however, it is evident that the inception of the bud begins by the division of certain cell at inner root cortex, and that the formation of the first leaf and root is taken place by the development of meristematic cells themselves.

3. Conclusion

On the basis of the foregoing discussion the intrageneric relationship among each genus, *Botrychium*, *Helminthostachys* and *Ophioglossum* may be concluded as follows.

According to the current system *Botrychium* may be divided into 3 subgenera; *Sceptridium*, *Eubotrychium* and *Osmundopteris*. In *Sceptridium* the rhizome shows the secondary thickening of xylem and development of the cork layer, the bundle being collateral and endarch. These facts prove that this plant is derived from the large and freely branched forms. The root is triarch or rarely polyarch. In *Eubotrychium* the rhizome resembles to that of the former, but the secondary thickening of the xylem is not found. The root is diarch or rarely triarch. These anatomical features show a stage of simplification or reduction from the former. In *Osmundopteris* the xylem of the rhizome resembles essentially to that of *Sceptridium*, but it is more developed. The root is tetrarch or rarely heptarch. Increase of the xylem groups of the root is far from the nature of other Ophioglossaceous ferns. This is also the same in *Helminthostachys*. Though it is fairly difficult to assign which is the most primitive subgenus among them, it may be said that *Sceptridium* is more primitive, since the relic of ancient features can be found anywhere.

In the second genus *Helminthostachys*, which is represented only by a single species, the rhizome is characterized by 1) creeping nature, 2) dorsiventrality, 3) single leaf trace, 4) less overlapping gaps, 5) mesarch xylem, 6) no secondary thickening, and 7) round or oval pitted tracheids.

The stele of the root is from hexarch to heptarch. The occurrence of mesarch condition in xylem of the rhizome as well as hexarch or heptarch condition in the root is so unusual that this genus should be transformed from other Ophioglossaceae, as pointed out in the previous paper.

The third genus *Ophioglossum* may be divided into 4 subgenera; *Euophioglossum*, *Ophioderma*, *Cheiroglossa* and *Rhizoglossum*, of which the last two have not been observed. In *Euophioglossum* the rhizome has slender and dictyostele, whose xylem is endarch. The metaxylem is composed of irregularly shaped reticulate tracheids and there is no secondary thickening. The endodermis is less obscure than that of other subgenera. The root is generally monarch. It is definitely suggested that this condition may be due to a reduction or simplification from the diarch one.

In *Ophioderma* the rhizome is radial in structure, and the stele is dictyostelic and shows an endarch protoxylem without secondary thickening. The root branches monopodially and the stele is triarch or tetrarch. This subgenus may be regarded as the most advanced and specialized group of the family from these features of the rhizome and roots.

The general conclusion is that the stelar structure of the rhizome in 3 genera of the Ophioglossaceae ranges from the ectophloic solenostele to perforated dictyostele. It seems that this family indicates the primitive characteristics or the most specialization. The stele of the root also ranges from monarch to polyarch. At any rate, the external and internal features of the rhizome and the root in *Helminthostachys* are most aberrant, while those in *Ophioderma* are most specialized and advanced.

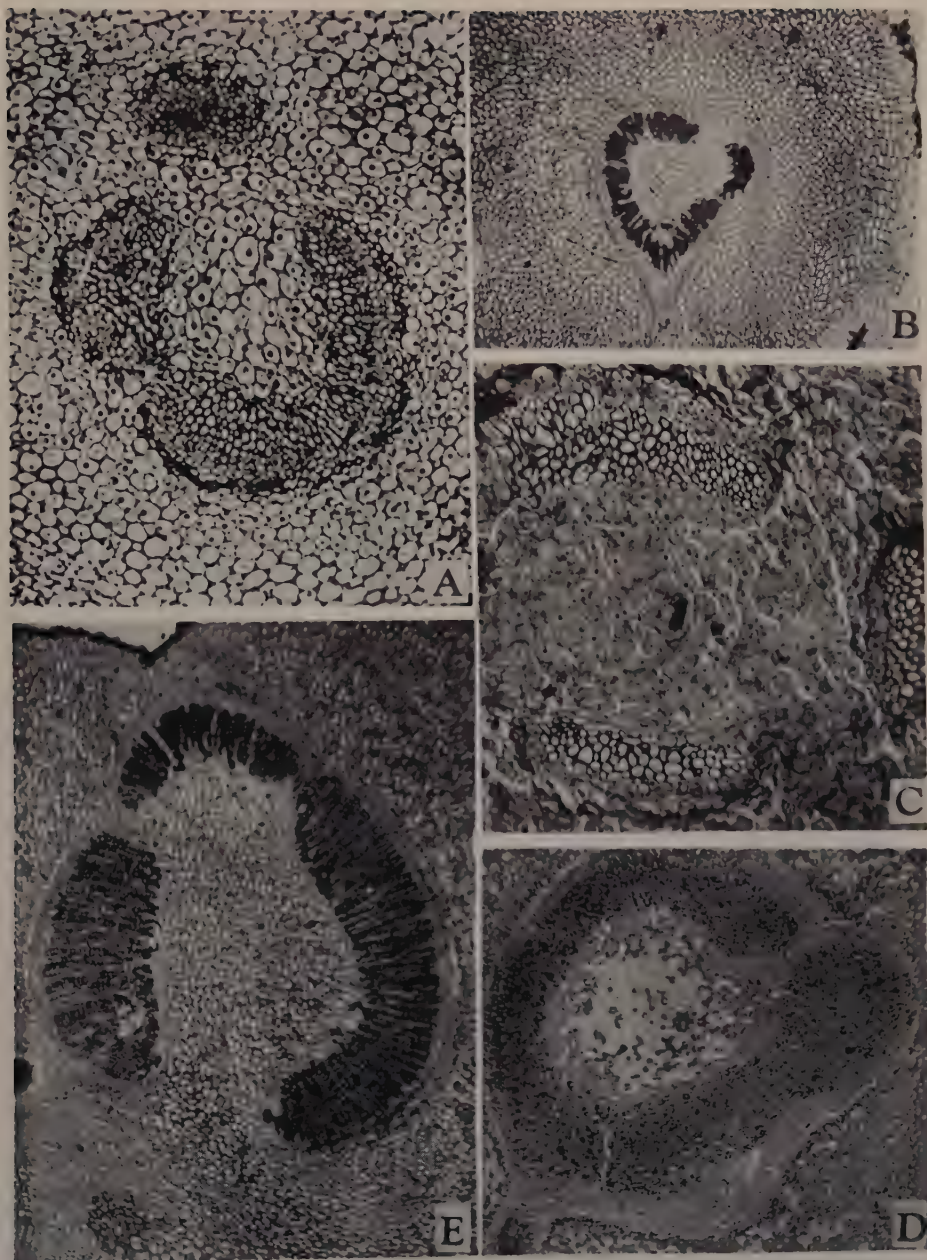
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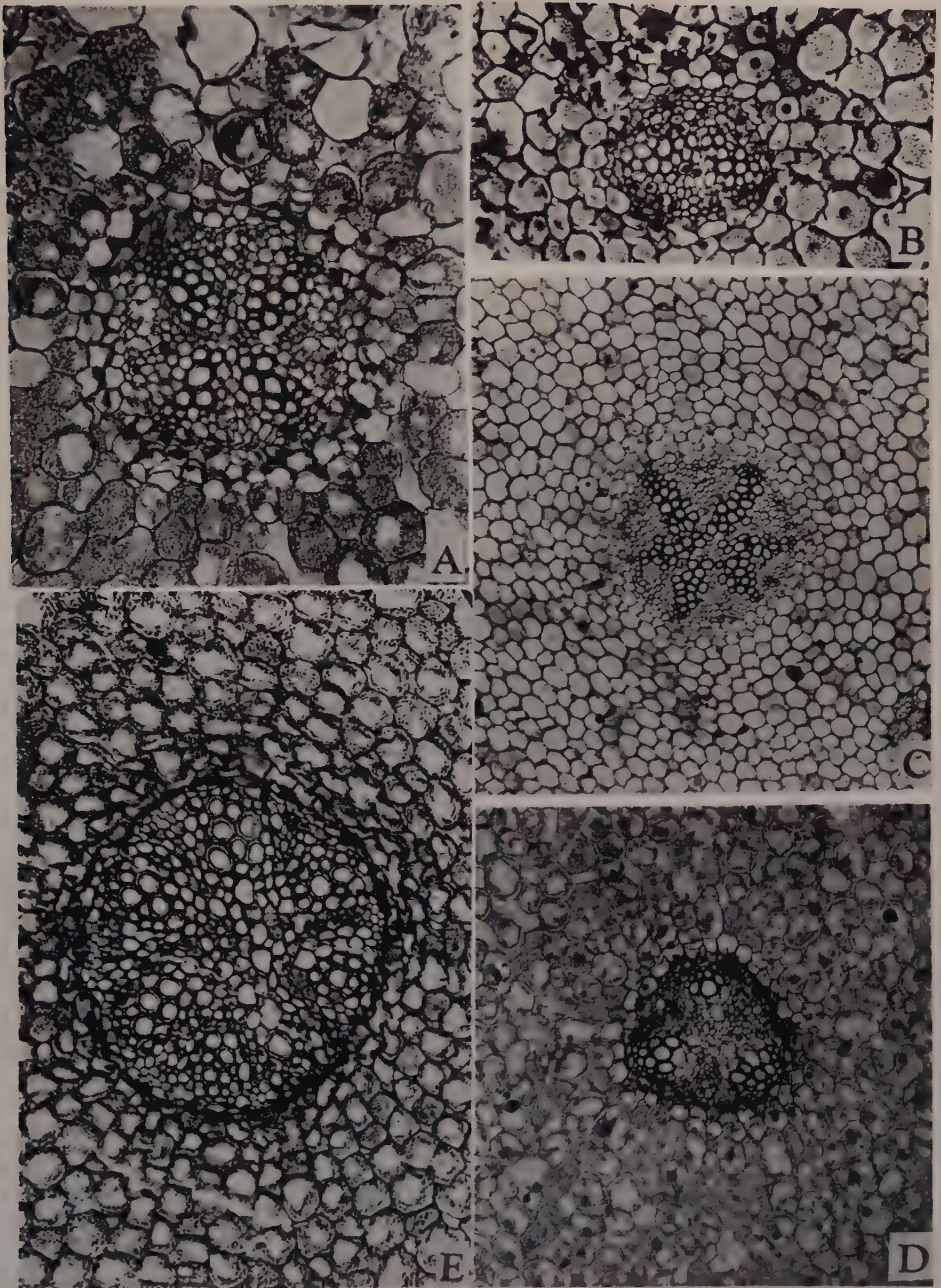
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Cross section of rhizomes. A, *Botrychium lanceolatum*. $\times 40$; B, *B. japonicum*. $\times 30$; C, *Ophioglossum littorale*. $\times 40$; D, *Helminthostachys zeylanica*. $\times 30$; E, *B. strictum*. $\times 30$.



Cross section of roots. A, *Botrychium japonicum*. $\times 50$; B, *B. Lunaria*. $\times 50$; C, *B. japonicum*. $\times 40$; D, *B. virginianum*. $\times 50$; E, *Helminthostachys zeylanica*. $\times 50$.

Simultaneous Measurement of Transcellular Osmosis and the Accompanying Potential Difference¹⁾

By

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Osmosis through an internodal cell of *Nitella*, occurring when one end of the cell is in contact with water and the other end with sucrose solution, has been first studied by Osterhout (1949). Kamiya and Tazawa (1956), who referred to the phenomenon as "transcellular osmosis," measured the volume of water transported by the osmosis with a high precision by means of a specially designed double-chamber volumeter. They analysed the phenomenon in detail and calculated the water permeability constants of the cell for both endosmosis and exosmosis.

Accompanying the transcellular osmosis, electric potential difference (P.D.) is produced between the external media on the endosmosis and exosmosis sides of the cell. Osterhout (1949, 1954) and one of us (Nishizaki, 1955) studied how P.D. thus generated changes in relation to transcellular osmosis. Nishizaki found that it is much dependent on the concentration of KCl in the surrounding media. When the concentration of KCl in the external media is not too low (0.05-0.01 M), the diffusion potential theory accounts for the time course of the changes in the P.D. between the endosmosis and exosmosis sides. When, however, the concentration of KCl is very low (less than 10^{-3} M), the P.D. referred to takes a time course different from that observed when its concentration is higher. Nishizaki is thus of the opinion that the change in mobility of K^+ relative to Cl^- is responsible for this phenomenon.

In order to elucidate the relation between osmosis and bioelectric phenomena, it is desirable to measure both osmosis and P.D. simultaneously. In the present work attempt has been made to read the volume of water transported through a single cell of *Nitella* from one end to the other and simultaneously the P.D. generated between the two ends. The experiments were conducted by combining the double-chamber volumeter modified from that used by Kamiya and Tazawa with the potentiometric circuit used by Nishizaki.

1. Material and Method

The material used was the internodal cell of *Nitella flexilis* supplied from the Botanical Garden of Kyoto University. The cells, 4-5 cm long, were freed and immersed in the experimental solution, either 10^{-2} M or 10^{-3} M KCl,

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for at least one day prior to the experiment.

The essential part of the apparatus used in this experiment is shown in Fig 1.

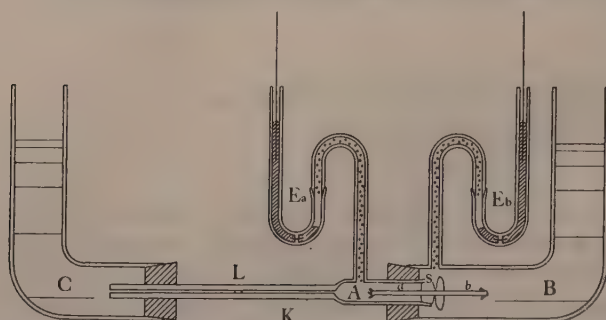


Fig. 1. The setup for measuring the transcellular osmosis and P.D. The two ends of an internodal cell *a* and *b* are placed in the small chamber *A* and thick bent tube *B* penetrating the partition cock *S*. *E_a*, *E_b*: calomel electrodes, *K*: capillary, *L*: index bubble, *C*: glass tube with water for compensating the difference in hydrostatic pressure between the two sides of the partition cock.

Between the small chamber *A* and the bent thick tube *B* there is a tiny partition cock *S* having a groove just sufficient for mounting the internodal cell. In the experiment the cell is brought in position in the groove with a small amount of vaseline. Under such a condition, there is no passage either for water or ions between *A* and *B* except through the cell. While tube *B* opens to the air, chamber *A*, with a capacity of 1 c.c., is connected with another bent thick tube *C* by means

of a fine capillary *K*. Tube *C* serves to keep the hydrostatic pressure equal on both sides of cock *S*. There is a tiny air bubble *L* in the capillary *K* with a bore of 0.6 mm. By this setup the volume of water transported through the cell from *A* to *B* or *vice versa* under the experimental conditions is exactly indicated by the shift of the air bubble, the position of which is measured by the aid of a travelling microscope. In order to measure the P.D. accompanying the osmosis, *A* and *B* are provided with calomel electrodes *E_a* and *E_b*, which are led to the potentiometer circuit.

In the experiment, *A* was filled always with KCl solution of 10^{-2} or of 10^{-3} M, while *B* was filled either with the same KCl solution as that in *A* or with the same KCl solution to which sucrose has been added. The main part of the setup which is shown in Fig. 1 is immersed in a water-bath with a constant temperature, except two openings of the tubes *B* and *C*, and two openings of the calomel electrodes *E_a* and *E_b*, which are left in the air, so that the shift of the bubble due to the temperature fluctuation is avoided. The temperature of the bath was kept constant at 20°C within the precision of $\pm 1/100^\circ\text{C}$. In order to avoid possible temperature disturbances when the solution in *B* is replaced, the solutions to be substituted are kept in advance in the flasks placed in the same water bath in which the apparatus was immersed.

Transport volume of water and the P.D. were measured by the two observers simultaneously at 1 or several minutes' interval. By plotting on a graph the two series of values thus obtained, we get two curves, one concerning osmosis and the other P.D. In the following figures, the transport volume of water is designated with a plus sign when the water flows from

A to B and with a minus sign when it flows in the opposite direction. The P.D. is expressed by the potential of B against that of A .

2. Experimental Results

When A and B are both filled with the same solution, no flow of water generally takes place through the cell, nor is there any P.D. between A and B . The material which behaved otherwise was discarded from the experiment. After the air bubble was checked to show no drift the KCl solution in B was replaced with the KCl solution to which sucrose has been added. The experiments were conducted under the following conditions.

(1) 0.01 M KCl (A) — 0.01 M KCl+0.2 M Sucrose (B)

The left half of Fig. 2 represents the results of the simultaneous measurement of both the transcellular osmosis (forward osmosis, *cf.* Kamiya and Tazawa, 1956) and the accompanying P.D. in 0.01 M KCl under the osmotic gradient of 0.2 M. It is noticed here that the P.D. follows a time course similar to that of osmosis which was studied by Kamiya and Tazawa in detail. After 6 minutes, by which time $1.18 \mu\text{l}$ water was carried from A to B through the cell, P.D. curve attained an equilibrium generating 16.5 mV between A and B .

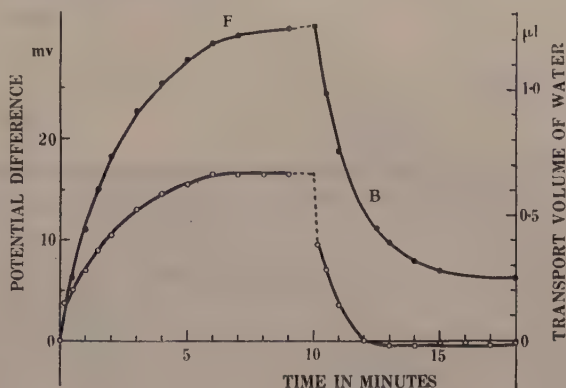


Fig. 2. Time course of transcellular osmosis (solid circles) and accompanying P.D. (open circles). F : forward osmosis, B : backward osmosis.

By substituting again 10^{-2} M KCl for the solution of 10^{-2} M KCl+0.2 M sucrose in B after 10 minutes, water started to move immediately in the reverse direction (backward osmosis). The P.D., too, changed in this case in parallel with osmosis as shown by the descending course of the curve. The reason why the curve of backward osmosis reaches an equilibrium before it comes down to the base line is well explained on the basis of kinetic analysis (Kamiya and Tazawa). This is, however, not the case in P.D. curves.

In Fig. 3 the parallelism between forward osmosis and P.D. is kept only around one minute at the beginning, as the P.D. curve soon takes a downward course. In the backward osmosis in the same figure, the P.D. drops far down to the negative side at first showing a parallelism with the osmosis curve. But it soon starts to ascend reaching its final equilibrium 14 minutes after backward osmosis began.

(2) 0.001 M KCl (A) — 0.001 M KCl+0.2 M Sucrose (B)

Figs. 4 and 5 show the results of the experiments under the same condition

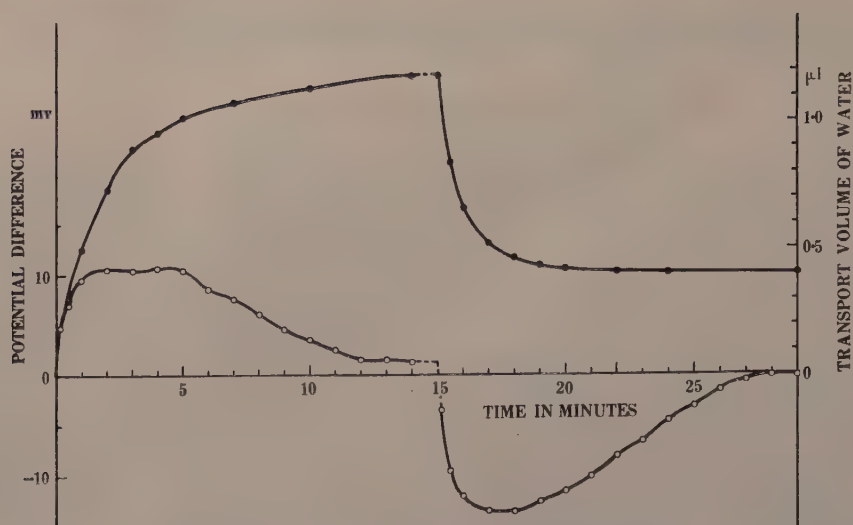


Fig. 3. Time course of transcellular osmosis (solid circles) and accompanying P.D. (open circles).

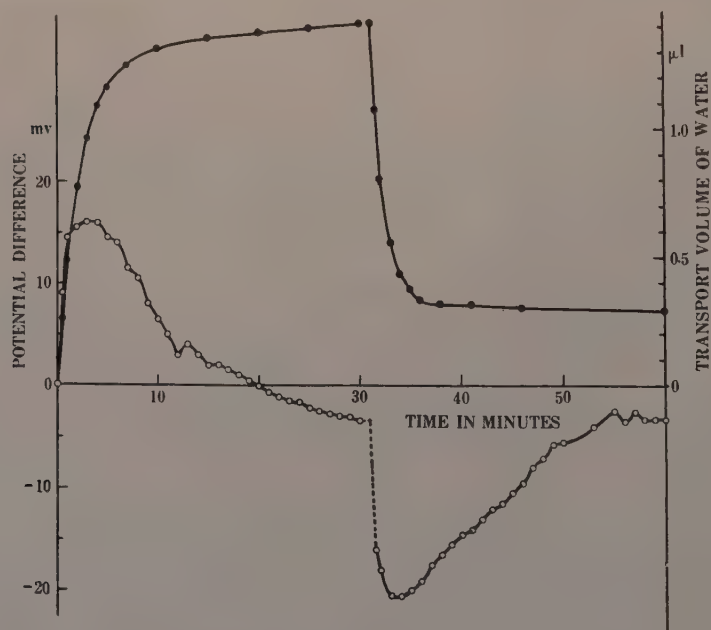


Fig. 4. Time course of transcellular osmosis (solid circles) and accompanying P.D. (open circles).

as that mentioned in section (1) except that the KCl concentration was 10^{-3} M. The curves of osmosis and P.D. in Fig. 4 are similar to those of Fig. 3: the

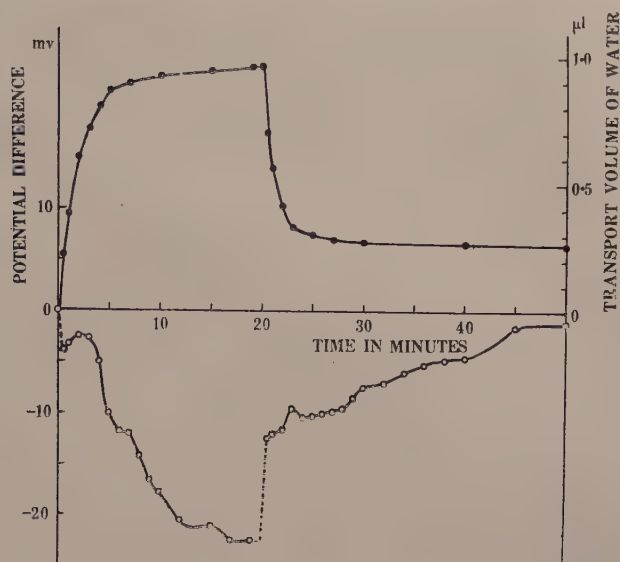


Fig. 5. Time course of transcellular osmosis (solid circles) and accompanying P.D. (open circles).

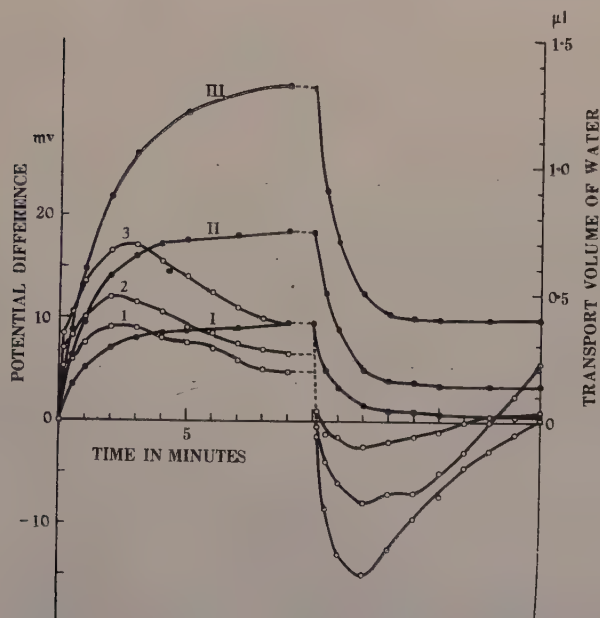


Fig. 6. Time courses of transcellular osmosis occurring under different osmotic gradients (solid circles) and accompanying P.D. Differences in osmotic pressure between the external solutions in A and B (*cf.* Fig. 1) are 0.1 M (curves I, 1), 0.2 M (curves II, 2) and 0.3 M (curves III, 3).

P.D. attains soon the maximum value before it falls down. But the P.D. turns to negative in Fig. 4 when it approaches an equilibrium, while it remains positive in Fig. 3. In Fig. 5, P.D. curve takes from the beginning a downward course on the whole. The final P.D. is far more negative as compared with that in Fig. 4. In the backward osmosis, the P.D. curve in Fig. 5 takes immediately an upward course without passing a falling phase as was observed in Figs. 3 and 4. The osmosis curves, on the other hand, display essentially identical features all the time.

(3) 0.01 M KCl (A) — 0.01 M KCl+(0.1–0.3) M Sucrose (B)

In order to see the effect of the intensity of osmosis to the P.D., a similar experiment was performed varying the sucrose concentration in B to 0.1, 0.2 and 0.3 M. Fig. 6 shows one of the results thus obtained. The concentration of KCl was 0.01 M in both A and B. Three pairs of curves of osmosis and P.D. took courses like those in Fig. 3. The same tendency was also observed under the osmotic gradient of as great as 0.6 M. Thus we see that the cell behaves essentially in the same manner under different osmotic gradients, though the intensity of osmosis and the magnitude of P.D. are naturally affected correspondingly. Similar results were obtained when 0.001 M KCl was used for the basic solution.

3. Analysis of the Results

Various theories have been presented for explaining bioelectric potential, among which the diffusion potential theory appears to be most adequate in many cases. Osterhout (1930) found that the P.D. across the protoplasmic layer in *Nitella* was proportional to the logarithm of KCl concentration in the surrounding medium within the range of 10^{-1} – 10^{-3} M. As a matter of fact, we can most reasonably explain the P.D. between the inside and outside of a *Nitella* cell in terms of diffusion potential. Admitting this is actually the case, how can we explain the behaviour of P.D. accompanying osmosis?

On the basis of the theory of diffusion potential, potentials of the media in chambers A and B in reference to the cell interior, E_a and E_b , are to obey the equation of Nernst and Henderson. Dominant ions in the *Nitella* cell responsible for diffusion potential being K^+ and Cl^- ³⁾, we can express the P.D. across the protoplasmic layer at *a* and *b* in the following equation:

$$E_a = \frac{RT}{F} \frac{U_K - V_{Cl}}{U_K + V_{Cl}} \ln \frac{Z_a}{Z_o} \quad (1)$$

$$E_b = \frac{RT}{F} \frac{U_K - V_{Cl}}{U_K + V_{Cl}} \ln \frac{Z_b}{Z_o} \quad (2)$$

3) The result of analysis by means of flame photometry (unpublished data of Kamiya and Kuroda) showed that, in *Nitella flexilis* which we used, there is as much as 0.1 M of potassium while sodium and calcium are 0.018 M and 0.008 M respectively. Thus most part of the P.D. between the interior and the exterior of the cell is due to the concentration difference in KCl between the interior and the exterior of the cell as well as to the difference in the mobilities of K^+ and Cl^- in the protoplasmic layer.

where U_K and V_{Cl} stand for the mobilities of K^+ and Cl^- ; R , T and F are gas constant, absolute temperature and Faraday's constant respectively; Z_a and Z_b are concentrations of KCl in a and b parts of the cell, and Z_o represents the concentration of KCl in the external solutions in A and B . Since KCl concentrations in A and B are regarded always to be equal to each other in the course of osmosis, the P.D. between a and b is expressed as follows:

$$E = E_b - E_a = \frac{RT}{F} \frac{U_K - V_{Cl}}{U_K + V_{Cl}} \ln \frac{Z_b}{Z_a} \quad (3)$$

In forward osmosis, water enters the cell at a and passes through the inside of the cell, and leaves it at b . Accompanying the flow of water, the solutes in the vacuole are carried with it from a to b . This flow causes dilution of the cell sap at a and concentration at b , i.e., intracellular polarization in respect to sap concentration. In backward osmosis occurring when the external osmotic gradient is removed, water enters the cell at b and leaves it at a , which naturally results in the depolarization of the sap concentration. The phenomenon is visualized by staining the vacuole with a proper dye, such as neutral red; in the forward osmosis, the dye is accumulated on the b side and diluted on the a side where water enters. In the backward osmosis, the dye tends to disperse homogeneously throughout the vacuole. As $\frac{Z_b}{Z_a}$ is regarded to be equal to the ratio of sap concentration at b and a , we can rewrite (3) in the form

$$E = E_b - E_a = \frac{RT}{F} \frac{U_K - V_{Cl}}{U_K + V_{Cl}} \ln \frac{C_b}{C_a} \quad (4)$$

where C_a and C_b are the concentration of the sap at a and b . If we assume that no leakage of solutes in the cell occurred during osmosis, C_a and C_b are expressed as functions of the volume of water (v) passing the cell (Kamiya and Tazawa, 1956). Namely, in forward osmosis;

$$\left. \begin{aligned} C_a &= C_0 e^{-\frac{v}{V_a}}, \\ C_b &= C_0 \left\{ 1 + \frac{V_a}{V_b} (1 - e^{-\frac{v}{V_a}}) \right\} \end{aligned} \right\} \quad (5)$$

and in backward osmosis;

$$\left. \begin{aligned} C_a &= C_{a_0} + C_{b_0} \frac{V_b}{V_a} (1 - e^{-\frac{v}{V_b}}), \\ C_b &= C_{b_0} e^{-\frac{v}{V_b}}. \end{aligned} \right\} \quad (6)$$

where C_0 is the initial concentration of the cell sap, V_a and V_b are the volumes of the cell parts a and b ⁴⁾, and C_{a_0} and C_{b_0} are the final osmotic pressures of the cell sap at a and b after forward osmosis reached equilibrium. The

4) The volume of the cell part mounted in partition cock (S) between A and B is included in part a , since it behaves like a in supplying solutes to b in the forward osmosis and in receiving them from b in the backward osmosis.

values of C_{a_0} and C_{b_0} in (6) are calculated from the following formula⁵⁾:

$$\left. \begin{aligned} C_{a_0} &= C_0 - (C_B - C_A) \frac{V_b}{V_0}, \\ C_{b_0} &= C_0 + (C_B - C_A) \frac{V_a}{V_0}. \end{aligned} \right\} \quad (7)$$

Substituting (5) and (6) in (4), we can express the P.D. accompanying forward and backward osmoses as the function of the volume of water (v)⁶⁾ carried.

By substituting C_{a_0} and C_{b_0} in (7) for C_a and C_b in (4), we can calculate E after transcellular osmosis reached equilibrium. According to Osterhout, $\frac{U_K - V_{Cl}}{U_K + V_{Cl}}$ amounts to nearly one, as the mobility of Cl^- (V_{Cl}) is much smaller than that of K^+ (U_K). Hence by assuming that $\frac{U_K - V_{Cl}}{U_K + V_{Cl}}$ remained at 1 in the course of osmosis and by substituting the values for the constants F , R and T , (3) and (4) are reduced to

$$E = 58 \log \frac{Z_b}{Z_a} = 58 \log \frac{C_b}{C_a} \quad (9)$$

where 293°C was taken for T as all experiments were performed around 20°C. E is expressed in this case in mV. In the material used for the experiment shown in Fig. 2 the whole length of the cell was 42 mm, in which a and b ends were both 21 mm long respectively. As area of cross section of the cell was nearly the same all over the cell, we have

$$\frac{V_a}{V_0} = \frac{V_b}{V_0} = \frac{21}{42} = \frac{1}{2} \quad (10)$$

The osmotic pressure of the cell sap C_0 was about 0.26 M, and the osmotic gradient ($C_B - C_A$) established by sucrose between the two chambers was 0.2 M. Hence the ratio $\frac{C_{b_0}}{C_{a_0}}$ is known to be $\frac{0.36}{0.16}$ from equations (7). Thus we obtain 20.3 mV from (4) for E in this experiment (Fig. 2) whereas the observed value was 16.5 mV. This shows that $\frac{U_K - V_{Cl}}{U_K + V_{Cl}}$ was as a matter of fact kept constant close to 1.

The causal sequences of the phenomena are shown diagrammatically in Fig. 7. At lower left are represented the time courses of forward (F) and backward (B) osmoses, which are derived from equation (8). These time

5) Kamiya and Kuroda (1956), who figured out experimentally the course of the change in the osmotic pressures at a and b during transcellular osmosis, showed that the values obtained by the experiment and C_{a_0} and C_{b_0} calculated from (7) coincide very satisfactorily as far as C_B was not more than 0.4 M. The value of C_B was less than 0.4 M in our present experiment.

6) The time course of v , which was determined empirically in this work, is expressed in the following formula derived by Kamiya and Tazawa.

$$v = V_a \ln \frac{1}{K_2} [K_1 - (K_1 - K_2) e^{-\frac{K_2 t}{V_a}}] \quad (8)$$

(8) is valid for the forward osmosis; for the backward osmosis V_b is to be substituted for V_a .

courses were obtained experimentally in the present work. At the upper left are shown changes in sap concentrations at *a* and *b* accompanying forward and backward osmoses. These curves are graphical representations of (5) and (6). Changes of sap concentrations at *a* and *b* (C_a and C_b) necessarily modify the resting P.D. between the interior and the exterior at *a* and *b* ends (E_a and E_b) according to (1) and (2). The situation is shown at upper right. The P.D. between *A* and *B* being ($E_b - E_a$), which we measured, we can predict its time course as such represented by curve *E* at lower right. This proved to be actually the case in Fig. 2.

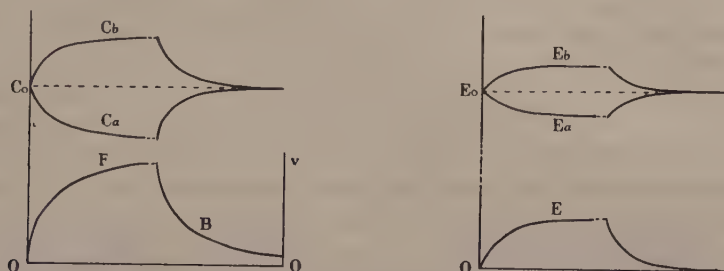


Fig. 7. Diagrammatic representation explaining the causal relation of osmosis (v), sap concentration (C_a , C_b), P.D. between the interior and exterior of the cell (E_a , E_b) and P.D. between the two external media in *A* and *B* (E).

Though the behaviour of the cell such as shown in Fig. 2 is favourably accounted for in terms of polar change in the concentration of the cell sap alone, there were also cases such as shown in Fig. 3 in which P.D. took a different mode of changes from Fig. 2 under the same experimental condition.

So far we have assumed $\frac{U_K}{V_{Cl}}$ to be constant during osmosis. If the assumption were true, the downward course of the P.D. shown in Fig. 3 would not be explained. Nishizaki (1955) suggested that this anomalous feature of change in the P.D. may be due to the change in $\frac{U_K}{V_{Cl}}$. There may be two possibilities for this, namely, either $\frac{U_K}{V_{Cl}}$ decreases on the exosmosis side, or it increases on the endosmosis side. But the latter does not come into question, since the maximum value of $\frac{U_K - V_{Cl}}{U_K + V_{Cl}}$ is 1 when its real value under normal condition is already nearly 1. Therefore the proper assumption to be made is that in the forward osmosis $\frac{U_K}{V_{Cl}}$ may decrease on the exosmosis side and not on the endosmosis side of the cell. In the backward osmosis, the P.D. curve takes a steep downward course keeping parallelism with the osmosis curve during the first few minutes, but the P.D. curve soon takes an upward course which is not the case in the osmosis curve. The steep downward course of the P.D. curve at the beginning is probably due to the concentration change resulting from the reverse flow of water. The following rising phase of the P.D. curve is to be understood if we assume the restoration of the relative mobility of K^+ at *b* end where water escaped in forward

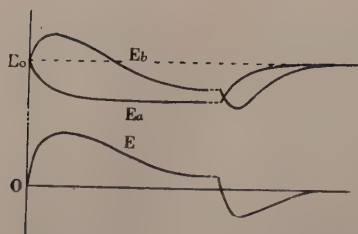


Fig. 8. Diagrammatic representation showing the hypothetical time courses of E_a and E_b , E , which was measured, is the difference between the two.

osmosis and now in backward osmosis it enters.

The situation is hypothetically elucidated in Fig. 8. The P.D. (E_a) between the interior and the exterior on a side shows the normal course as is represented by the curve E_a at the upper right in Fig. 7. On the other hand, E_b in Fig. 8 takes a course different from E_b in Fig. 7, as $\frac{U_K}{V_{Cl}}$ is assumed to decrease gradually in forward osmosis. In backward osmosis $\frac{U_K}{V_{Cl}}$ is assumed to restore its original value of 1.

In Fig. 4 the P.D. curve comes down to the negative side showing a more conspicuous tendency to descend. We are inclined to believe from this fact that decrease of relative mobility of K^+ might be more pronounced when the cell is in 10^{-3} M KCl than when it is in 10^{-2} M KCl. But a simple mathematical treatment proves that P.D. is lower in a more diluted KCl solution than in a KCl solution of higher concentration even when the change in $\frac{U_K}{V_{Cl}}$ remains the same. As has been shown before, the course of P.D. in Figs. 2 and 3 are different even though the experimental conditions were the same. Similarly, those in Figs. 4 and 5 are also different according to the cells used. The fact being such, we must conclude that there is a considerable difference in the mode of change in $\frac{U_K}{V_{Cl}}$ according to the specimen used. So far as our present knowledge goes, there is no need to assume $\frac{U_K}{V_{Cl}}$ to be dependent on the KCl concentration in the external medium.

Fig. 6 shows time courses of the change in P.D. in one and the same cell when the sucrose concentrations at B were 0.1, 0.2 and 0.3 M. We see in the figure that the tendency of the P.D. curve to take a downward course is the more pronounced the greater the intensity of osmosis is. The P.D. generated in forward osmosis is lower in each case than the theoretical value which is expected when the mobility on the b side (exosmosis side) remained unchanged. The deviation of the P.D. obtained experimentally from the one obtained theoretically is expressed in the following formula;

$$E_{thor} - E_{exp} = \frac{RT}{F} \ln \frac{Z_b}{Z_0} - \frac{RT}{F} \left(\frac{U_K - V_{Cl}}{U_K + V_{Cl}} \right)_b \ln \frac{Z_b}{Z_0} \\ = 58 \log \frac{Z_b}{Z_0} \left\{ 1 - \left(\frac{U_K - V_{Cl}}{U_K + V_{Cl}} \right)_b \right\}. \quad (11)$$

The KCl concentration in the cell sap being about 0.1 M we have

$$Z_a = 0.1 \times \frac{C_a}{C_0}, \quad Z_b = 0.1 \times \frac{C_b}{C_0}.$$

Substituting (7) in the above formula, we get the final concentration of KCl on a and b side (Z_{a_0} and Z_{b_0}) as follows:

$$Z_{a_0} = 0.1 \left(1 - \frac{C_B - C_A}{C_0} \frac{V_b}{V_0} \right), \quad (12)$$

$$Z_{b0} = 0.1 \left(1 + \frac{C_B - C_A}{C_0} \frac{V_a}{V_0} \right). \quad (13)$$

In forward osmosis the theoretical value of P.D. (E_{theor}) can be obtained by substituting (12) and (13) in (9). Since in this experiment (Fig. 6) we have $\frac{V_a}{V_0} = \frac{26}{44}$, $\frac{V_b}{V_0} = \frac{18}{44}$, $C_0 = 0.26$ M, and $C_B - C_A = 0.1, 0.2$ and 0.3 M, E_{theor} under the osmotic gradients of $0.1, 0.2$ and 0.3 M will be $9.35, 18.8$ and 28.7 mV. On the other hand the experimental values of E , which were obtained 9 minutes after forward osmosis started, were $4.5, 6.5$ and 9.5 mV. Hence, $E_{theor} - E_{exp}$ in (11) are $4.85, 12.3$ and 19.2 mV when $0.1, 0.2$ and 0.3 M sucrose were admitted in B . In this experiment the concentration of KCl (Z_0) in the external media in A and B was 10^{-2} M and Z_{b0} is to be obtained from (13). Thus the term $58 \log \frac{Z_b}{Z_0}$ in (11) proved to have the values of $63, 67.3$ and 71.3 mV, and hence $\frac{U_K - V_{cl}}{U_K + V_{cl}}$ in (11) is calculated to be $0.92, 0.83$ and 0.73 , when the osmotic gradients between A and B were $0.1, 0.2$ and 0.3 M respectively. We, therefore, find $\frac{U_K - V_{cl}}{U_K + V_{cl}}$ to be the less the more intense the osmosis is. In other words, $\frac{U_K}{V_{cl}}$ decreases when the rate of osmosis increases. In case when 10^{-2} M KCl was used as basic solution, a similar relation was obtained.

According to Osterhout, the value of $\frac{U_K}{V_{cl}}$ is constant in the range of $10^{-1} - 10^{-5}$ M KCl in the external medium. This conclusion may be true in the cell which shows no osmosis. The results of experiments, on the other hand, were reasonably explained under the assumption that $\frac{U_K}{V_{cl}}$ decreased during the course of transcellular osmosis at the cell part where water escaped. $\frac{U_K}{V_{cl}}$ is not necessarily dependent on the concentration of KCl in the outer medium, but is dependent on the intensity of osmosis. The problem as to what mechanism is responsible for the change in $\frac{U_K}{V_{cl}}$ will await further study.

4. Summary

(1) When the two halves of an internodal cell of *Nitella* are brought in contact with solutions having different osmotic pressures, water moves through the cell from that half having lower osmotic pressure to the other having higher osmotic pressure (transcellular osmosis). In the present work the new apparatus was constructed which enabled the simultaneous measurements of transcellular osmosis and the accompanying P.D. (electric potential difference) in an internodal cell of *Nitella*.

(2) Transcellular osmosis brings about an intracellular concentration difference which induces the P.D. between the two ends of the cell.

(3) The osmotic behaviour of the cell was always the same as to satisfy the kinetic equation presented by Kamiya and Tazawa (1956). On the other hand the time course of the P.D. accompanying transcellular osmosis not always showed parallelism with the latter.

(4) The change in P.D. unparallel to the osmosis is comprehensible

under the assumption that the mobility of K^+ relative to Cl^- ($\frac{U_K}{V_{Cl}}$) decreases at one end of the cell, where water goes out and hence the concentration of the cell sap increases.

(5) The change in $\frac{U_K}{V_{Cl}}$ is the more pronounced the more intense the osmosis is.

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Studies on the Metabolism of Molecular Hydrogen in Microorganisms

I. Oxyhydrogen reaction in the cell-free enzyme system extracted from *Azotobacter*

By

Yoshiharu ODA*, Takahiro MASUDA and Masataka HIGUCHI

(Received August 31, 1955)

The hydrogenase system in *Azotobacter* has been investigated particularly in connection with its possible significance for nitrogen fixation, because of the facts that the active hydrogenase can be observed only in the nitrogen fixing cells of *Azotobacter* (1) and the molecular hydrogen inhibits specifically the nitrogen fixation (2, 3).

In 1953, Wilson and his coworkers (4, 6) found that the oxyhydrogen reaction can take place in crude juice of *Azotobacter vinelandii*, accompanied by the esterification of phosphoric acid through adenosine 5'-phosphoric acid.

Hydrogenase is easily inactivated by oxygen both in cell-free systems and in the whole cells (5, 6, 7). Nevertheless, the enzyme can be also found in *Azotobacter* grown in N-free medium under the strictly aerobic condition. Furthermore, in the oxyhydrogen reaction, which may be carried out by the enzyme system containing hydrogenase, oxygen functions as a substrate at one terminal side.

In the present investigation, the oxyhydrogen reaction has been studied with the cell-free enzyme systems extracted from *Azotobacter*, with particular reference to the interrelation between nitrogen fixation and hydrogenase, and to the significance of the paradoxical presence of hydrogenase in a strict aerobe, *Azotobacter*.

Experiments

Measurements of oxyhydrogen reaction and hydrogenase activity—Measurements of oxyhydrogen reaction and hydrogenase activity were carried out by the ordinary method using Warburg's manometer.

In the latter case, methylene blue was added in the preparation as the hydrogen acceptor. Air in the manometric vessels was replaced with a gas mixture of 75% of H_2 and 25% of air, in the measurement of oxyhydrogen reaction. But the mixing ratio was changed according to the experimental object.

Preparations of the cell-free extracts—Cells of *Azotobacter chroococcum* grown in the Burk's N-free medium for 72 hrs. were harvested and washed

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thrice with the distilled water. The cell-paste was ground with the same weighed quartz sand at a lower temperature than 4 and then extracted with M/10 phosphate buffer solution (pH 7.0), the volume of which was in proportion of 3 ml. per gr. of cell-paste. The mixture was centrifuged at 2,000 g for 20 minutes and the supernatant was called prepn. A.

Prepn. B was the supernatant obtained from prepn. A by centrifuge at 11,000 g for 15 minutes, while prepn. C was the Suspension of the sediment in M/10 phosphate buffer. The supernatant obtained from prepn. B by further centrifuge at 22,000 g for 20 minutes was designated as prepn. D. Prepn. C was the particle fraction, probably corresponding to mitochondrial one of animal tissues. It was brownish red in color. Smaller particles and soluble proteins might be contained in prepn. D.

Results

Oxyhydrogen reaction by the cell-free preparation—The oxyhydrogen reaction was observed with prepn. A, B and C (Fig. 1). The activity of prepn. A was a little higher than those of prepn. B and C. The activities of the latter two were almost the same. No activity was observed with prepn. D, although the hydrogenase activity could be observed. Hydrogenase in prepn. D might be free and soluble one, which was probably liberated from particles by the procedures of extraction. It was less stable for overnight storage in refrigerator than that in prepn. C. This fact might suggest that the enzyme bound to the particles is more stable against the inactivation by oxygen than the free one.

Even if a considerable gas uptake was initially observed in 100% of H_2 (Fig. 2), it might imply the contamination of small amounts of O_2 due to the

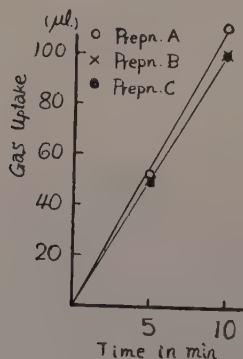


Fig. 1. Comparison of oxyhydrogen reaction activity of each preparation.

2.0 ml. of prepn. A, B and C, were used. Partial pressure of O_2 in gas mixture was 5%. Temp. 30°C, pH 7.0.

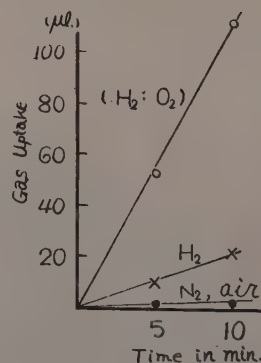


Fig. 2. Oxyhydrogen reaction by cell-free preparation.

2.0 ml. of prepn. B was used. ($H_2: O_2$); gas mixture of 25% of air (5% O_2) and 75% of H_2 . Temp. 30°C, pH 7.0.

imperfection of the gas exchange of vessels or the presence of certain hydrogen acceptors in the preparation.

Effect of partial pressures of oxygen—Effect of partial pressures of oxygen upon the oxyhydrogen reaction are shown in Fig. 3. Activity of the oxyhydrogen reaction in 10% of O_2 was approximately a quarter of that in 5%. This effect might be due to the oxygen inhibition of hydrogenase in the system. But it is extremely interesting that the oxyhydrogen reaction having oxygen as one of substrates is still inhibited by oxygen. It has been shown as given in Fig. 4 that such the lowering of activity was remarkably restored by addition of the small amounts of succinate. The broken line in the figure represents the estimated gas uptake, which can be obtained by the summation of the oxygen consumption due to the oxidation of the added succinate and the gas uptake caused by the oxyhydrogen reaction. If the oxyhydrogen reaction had taken place independently of the oxidation of

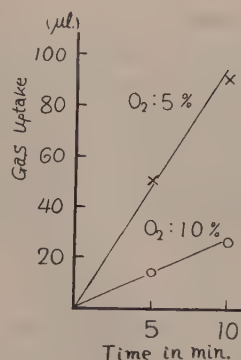


Fig. 3. Effect of partial pressures of oxygen upon the oxyhydrogen reaction.

1.0 ml. of prepn. B was used. (O_2 : 5%); 25% of air and 75% of H_2 . (O_2 : 10%); 50% of air and 50% of H_2 . Temp. 30°C, pH 7.0.

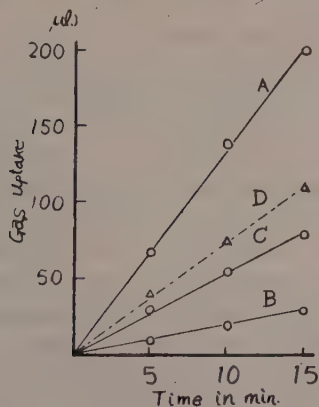


Fig. 4. Effect of succinate upon the oxyhydrogen reaction in the presence of 10% of O_2 .

1.0 ml. of prepn. B was used.

A; 0.2 ml. of M/10 succinate was added in an atmosphere of H_2 and air (10% O_2).

B; control, in an atmosphere of H_2 and air (10% O_2).

C; 0.2 ml. of M/10 succinate was added in an atmosphere of N_2 and air (10% O_2).

D; theoretical values. See the text.

Temp. 30°C, pH 7.0.

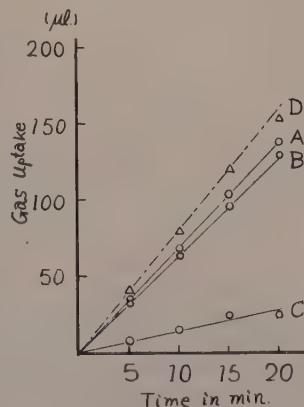


Fig. 5. Effect of succinate upon the oxyhydrogen reaction in the presence of 5% of O_2 .

1.8 ml. of prepn. B was used.

A; 0.2 ml. of M/10 succinate was added in an atmosphere of H_2 and air (5% O_2).

B; control, in an atmosphere of H_2 and air (5% O_2).

C; 0.2 ml. of M/10 succinate was added in an atmosphere of N_2 and air (5% O_2).

D; theoretical values. See the text.

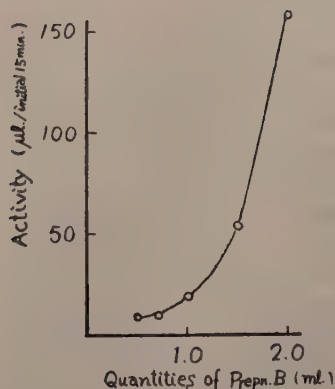


Fig. 6. Non-proportionate effect of dilution.

Different quantities of prepn. B diluted to the same final volume (2.0 ml.) with M/10 phosphate buffer were used.

Activity was gas uptake for initial 15 minutes.

Temp. 30°C, pH 7.0.

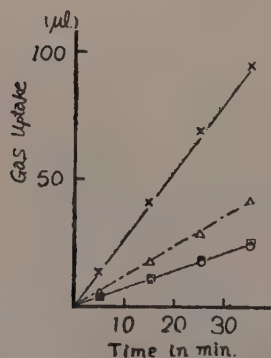


Fig. 7. Effect of succinate upon the diluted preparations.

0.5 ml. of prepn. B diluted to 2.0 ml. with M/10 phosphate buffer was used. Temp. 30°C, pH 7.0.

×; 0.2 ml. of M/50 succinate was added in an atmosphere of H_2 and air (5% O_2).

•; control, in an atmosphere of H_2 and air (5% O_2).

○; 0.2 ml. of M/50 succinate was added in an atmosphere of N_2 and air (5% O_2).

□; 0.2 ml. of succinate and 0.1 ml. of M/10 malonate were added in an atmosphere of H_2 and air (5% O_2).

Δ; theoretical values. See the text.

succinate, actual gas uptake would have accorded with the estimated value. It is sure, therefore, that the enzyme systems which had been inactivated by oxygen were reactivated by the addition of succinate. In 5% of O_2 , the added succinate had almost no effect upon the oxyhydrogen reaction (Fig. 5). Therefore, 5% of O_2 in the gas mixture would be the optimal pressure in the present condition.

Dilution effect and succinic reactivation—When the activities of the different quantities of prepn. B diluted to the same final volume (2.0 ml.) were measured, it was observed that the decrease of activity did not proceed in proportion to the amounts of the enzyme (Fig. 6). In this case, the activity was expressed by the amounts of the gas uptake for initial 15 minutes.

Although it is difficult to explain the cause of the dilution effect, it may be different from the dilution effect which was reported by H. Gest in the case of formic hydrogenlyase and dehydrogenase (8), because it appears to be caused rather by the action of oxygen solved in the buffer solution than by the lack of certain cofactors in the systems. When the small amounts of succinate was added to the preparation diluted to be very feeble in the activity of the oxyhydrogen reaction, the remarkable reactivation was observed as well (Fig. 7). The lower limit of the effective amounts of the added succinate was $4 \mu M$ per vessel in the present condition.

It is impossible to regard this effect as the stimulation of the succinic oxidation, since the gas uptake went on over the theoretical amount of oxygen consumed by the complete oxidation of $4 \mu M$ of succinate.

The similar reactivation was observed in the preparation inactivated by over-night storage in refrigerator, but it is obscure whether it is essentially identical with the effect of succinate on the dilution effect or not.

The reactivation of the diluted preparation by the addition of small amounts of succinate was inhibited by the adding of malonate (Fig. 7). When malonate was added to the mixture 5 minutes after the addition of the succinate, typical inhibition was observed, but, on the contrary, it had no effect on the reactivation when it was added 20 minutes later (Fig. 8). It was not caused by the particular property of the malonic inhibition, because the time delay of the addition of malonate had no influence on the inhibition of the succinic oxidation (Fig. 9).

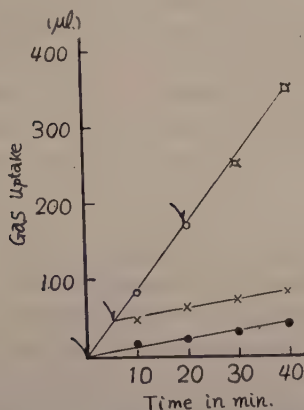


Fig. 8. Inhibition of succinic reactivation by adding of malonate. 1.0 ml. of prepn. B diluted to 2.0 ml. with M/10 phosphate buffer was used. 0.2 ml. of M/50 succinate and 0.1 ml. of M/10 malonate were added. Temp. 30°C, pH 7.0. Malonate was added at the arrow points.

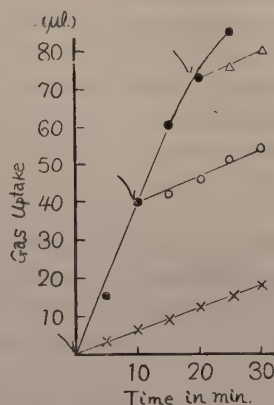


Fig. 9. Inhibition of succinic oxidation by adding malonate. 0.2 ml. of prepn. B, 0.1 ml. of M/10 succinate and 0.2 ml. of M/10 malonate were used. Gas phase; air. Malonate was added at the arrow points.

These results suggest that the succinic dehydrogenase system is involved in the mechanism of the reactivation and plays a rôle only in the initial period of the reactivation.

Effect of various substances on the dilution effect—The effect of the various substances on the dilution effect is shown in Table 1.

Succinate, methylene blue, Nile blue and toluidine blue were noticed to be effective on the reactivation of the diluted preparation. Among the dicarboxylic acids in TCA-cycle, only succinate was effective. The preparation inactivated by the dilution was not reactivated by the additions of fumarate, malate and oxalacetate, though the preparation weakly oxidized them except oxalacetate.

Reducing agents, for example cysteine and hydrosulfite, were completely non-effective, but it is impossible to conclude that the oxidation-reduction and oxygenation-deoxygenation in the hydrogenase system have entirely no connection with the inactivation and reactivation of the preparation, since

Table 1. Effect of various substances on the dilution effect.

Substances added	Gas uptake for initial 15 min.		
	Before addition (A)	After addition (B)	B/A
Succinate (4 μ M)	3.3	68.5	19.8
Fumarate (10 μ M)	5.1	5.9	1.2
Malate (10 μ M)	2.9	4.1	1.4
Oxalacetate (10 μ M)	1.0	2.2	2.2
Methylene blue (1 μ M)	3.1	54.0	17.4
Nile blue (1 μ M)	3.0	46.4	15.5
Toluidine blue (0.05%)	2.8	14.0	5.0
Methylviologen (1 μ M)	2.3	2.6	1.1
Cysteine (10 μ M)	2.2	2.5	1.1
Hydrosulfite (10 μ M)	2.3	2.3	1.0

the fact that the activity of the hydrogenase was reactivated in the presence of hydrosulfite was reported by Rittenberg and his coworkers (6, 7).

The oxido-reductive dyes except methylviologen were effective on the reactivation of the diluted preparations, but the mechanism of the reactivation appears to be considerably different from that of the succinic reactivation. The oxyhydrogen reaction was probably carried out by the catalytic action of these dyes directly linked with the enzyme hydrogenase. The enzyme which remained partially in active form might be enough to display a large gas uptake, because of the efficient catalytic action of these dyes.

Furthermore, no reactivation was noticed by adding of egg albumin

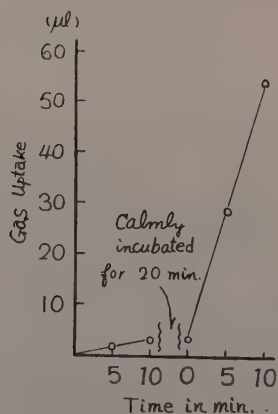


Fig. 10. Effect of calm incubation.
0.5 ml. of prepn. B diluted to 2.0 ml. with M/10 phosphate buffer was used.
Temp. 30°C, pH 7.0.

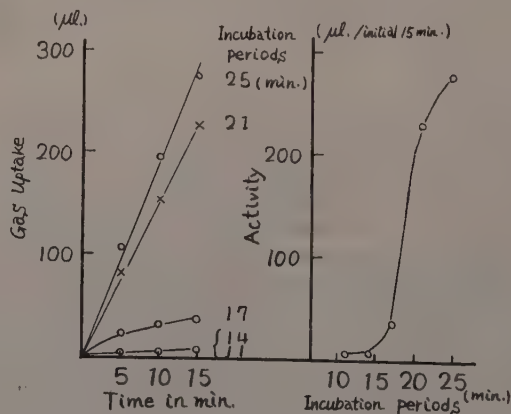


Fig. 11. Effect of incubation periods upon the reactivation.
1.0 ml. of prepn. B diluted to 2.0 ml. with M/10 phosphate buffer was used. Temp. 30°C, pH 7.0.

which had been found to be effective on the activation of the hydrogenase preparation diluted.

Effect of calm incubation on the diluted preparations—When the manometers containing the diluted preparations were incubated calmly without shaking for about 20 minutes, the activity of the oxyhydrogen reaction was markedly restored (Fig. 10). The anaerobic condition might be important to the reactivation of the enzyme inactivated by oxygen. The relation between the restored activity and the incubation periods are illustrated in Fig. 11.

Activity was defined to be whole gas uptake for initial 15 minutes. As can be seen in Fig. 11, it is noticed that a kind of threshold value in the incubation periods required to reactivate the diluted preparation is about 20 minutes. Molecular hydrogen was effective on the reactivation as a gas phase during the calm incubation but nitrogen was not (Fig. 12). Molecular hydrogen may be essentially required for the reactivation by the calm incubation.

It is conceivable that the active enzyme remained in a few amounts in the diluted preparation might, in the presence of hydrogen, reactivate the enzyme which was involved in that preparation and was inactivated by oxygen. As noticed in Fig. 11, therefore, the reaction might proceed along

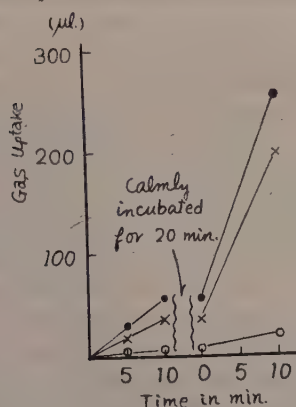


Fig. 12. Effect of gas phase during incubation.

1.0 ml. of prepn. B diluted to 2.0 ml. with M/10 phosphate buffer was used.

- ; incubated and measured in an atmosphere of H_2 and air (5% O_2).
- ×; measured in an atmosphere of H_2 and air (5% O_2) after incubation in H_2 .
- ; measured in an atmosphere of H_2 and air (5% O_2) after incubation in N_2 .

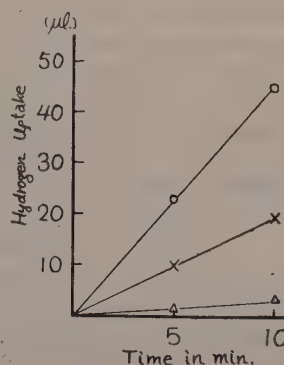


Fig. 13. Effect of anaerobic incubation upon the hydrogenase activity. Cells of *Azotobacter chroococcum* grown in ammonium medium for 48 hours was washed and suspended in M/10 phosphate buffer. The cell-suspension was anaerobically incubated for 4 hours in a medium containing 100 ml. of M/10 phosphate buffer, 1 g. of glucose, 0.05 g. of $MgSO_4 \cdot 7H_2O$, 0.02 g. of $CaCl_2$, 0.2 g. of $(NH_4)_2HPO_4$ and 0.2 g. of glutamate.

- ; incubated in N_2 .
- △; incubated in air.
- ×; not treated.

Activity was measured with the cell-suspension containing 0.112 mg. N per vessel.

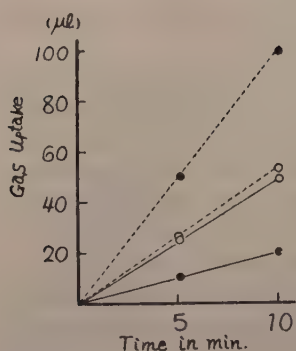


Fig. 14. Comparison of the cells anaerobically incubated with those grown in N-free medium; Activity was measured with the cell-suspension containing 0.112 mg. N per vessel.

---●---; Oxyhydrogen reaction by the cells grown in N-free medium.

---○---; Hydrogenase activity by the cells grown in N-free medium.

—○—; Hydrogenase activity by the cells anaerobically incubated.

—●—; Oxyhydrogen reaction by the cells anaerobically incubated.

an S-shaped curve characteristic of an auto-catalytic one.

Restoration of hydrogenase by anaerobic incubation—A complete restoration in the activity of hydrogenase was observed, when the cells of *Azotobacter* grown in ammonium medium were anaerobically incubated for 4 hours in the medium containing 1% of glucose, 0.2% of glutamate, 0.2% of ammonium phosphate and the other inorganic salts (Fig. 13). Activities of the cells grown in the N-free medium and of the cells anaerobically incubated are shown in Fig. 14.

The comparison of the cells anaerobically incubated with those grown in the N-free medium showed that the activity of hydrogenase was almost the same in both kinds of cells but the activity of oxyhydrogen reaction was extremely less in the former cells than in the latter.

Discussion

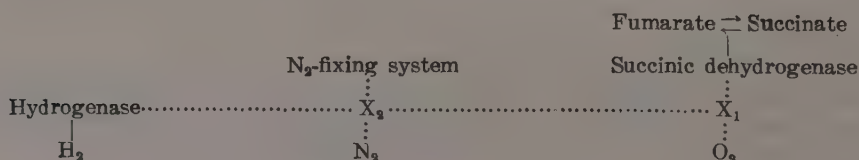
It is the most essential property of the hydrogenase that the enzyme is sensitive to oxygen and easily inactivated by it.

This property was observed even in the oxyhydrogen reaction which has oxygen as one of the substrates. In spite of that, the enzyme can be found in aerobic bacterium, *Azotobacter*

grown in N-free medium though it is mostly distributed in anaerobic bacteria. Therefore, there must be a certain mechanism to protect the enzyme from oxygen in *Azotobacter*. It seems that functional coupling of hydrogenase with succinic dehydrogenase system may be an instance of such protecting mechanism.

Rittenberg and his coworkers reported that the hydrogenase inactivated by oxygen could be reactivated by adding of $\text{Na}_2\text{S}_2\text{O}_4$, linking of glucose oxidase or merely degassing (6, 7). But the linking of glucose oxidase with hydrogenase was artificially observed only in the reconstructed system.

On the basis of the results mentioned above, a tentative mechanism of the succinic reactivation can be proposed:



In diagram, X_1 and X_2 are tentative components, which may take part in electron transport. Particularly X_2 is imagined to be also concerned with nitrogen fixation mechanism.

Oxyhydrogen reaction may be carried out through hydrogenase, X_2 and X_1 . Hydrogenase is easily inactivated in the presence of oxygen. But if oxidation of succinate takes place, hydrogenase is probably cut off from oxygen and reactivated by the electron derived from succinate.

The linkage of hydrogenase system with succinic dehydrogenase system is thought to bring about a competition between oxyhydrogen reaction and the oxidation of succinate. Actually that was supported by the preliminary experiment. When succinate was added to the diluted preparation, the amounts of succinate oxidized in an atmosphere of H_2 and air (5% O_2) were approximately one third of those in an atmosphere of N_2 and air (5% O_2).

When *Azotobacter* is grown in ammonium medium, lowering of hydrogenase activity may be caused by the lack of X_2 , since the protecting mechanism is lost by the incompleteness of the pattern.

It might be supported by the fact that the anaerobic incubation of the cells of *Azotobacter* grown in ammonium medium could restore the hydrogenase activity but not the oxyhydrogen reaction.

Stiffler and Gest found that the activity of hydrogenase, in the case of *Rhodospirillum rubrum* was observed in both cells from glutamate medium and from ammonia medium but photoevolution of hydrogen only in cells from glutamate medium (9). This interrelation between hydrogenase and photoevolution might be explained with the scheme mentioned above.

Actual determinations of these tentative components and final confirmation of the proposed mechanism are left in further investigation.

Finally the authors wish to express their gratitude to Prof. K. Okunuki for his kind support in the research work.

Summary

1. Oxyhydrogen reaction has been studied in cell-free preparations extracted from *Azotobacter chroococcum*, with particular reference to the interrelation between nitrogen fixation and hydrogenase. Activity was found wholly in the particle fraction, probably corresponding to the mitochondrial one of animal tissues.

2. Oxyhydrogen reaction having O_2 as one of substrates was inhibited by oxygen itself. But the inhibition could be excluded by adding of small amounts of succinate.

3. When the activities of different quantities of prepn. B diluted to the same final volume (2.0 ml.) were measured, it was observed that the decrease in activity did not proceed in parallel with the amounts of enzyme. But the non-proportionate lowering of activity was restored by adding of small amounts of succinate. Such the reactivations were inhibited by addition of malonate only in the initial period.

4. Succinate, methylene blue, Nile blue and toluidine blue were effective

on the reactivation.

5. When the diluted preparations were calmly incubated for 20 minutes in an atmosphere of hydrogen with air and of hydrogen, the activity of the oxyhydrogen reaction was markedly restored.

6. A complete restoration of hydrogenase was observed, when the cells of *Azotobacter* grown in ammonium medium were anaerobically incubated in the medium containing 1% of glucose, 0.2% of glutamate, 0.2% of ammonium phosphate and the other inorganic salts for 4 hours. The comparison of the cells anaerobically incubated with those grown in the N-free medium showed that the activity of hydrogenase was almost the same in both kinds of cells but the activity of oxyhydrogen reaction was extremely less in the former cells than in the latter.

7. Finally, on the basis of the results obtained, tentative mechanism of the inactivation by oxygen and of reactivation by succinate are proposed and discussed in relation to nitrogen fixation.

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Studies on the Georeaction shown in the Axes of some Herbaceous Plants

By

Ichiro SATO

(Received August 31, 1955)

If a vertical axis is forcibly fixed in the horizontal position, it loses its nature of a radial symmetry and comes to show remarkable differences in the anatomical structures as well as in the growth in thickness between its upper and lower sides (1, 4, 5, 6, 7, 8, 10, 11, 12). This phenomenon will be considered as a result of an unequal distribution of growth hormones due to the fact that gravity is now led to act on the plant axis not longitudinally, but transversally (2, 3, 13). The present studies were carried out for the purpose of obtaining the histological evidences for the inference stated above.

The author is heartily grateful to Professor Fuyuo Kagawa and Professor Shun-ichiro Imamura for their kind guidance and valuable criticism.

Materials and methods

The axes of jute plants and hemp plants which are now in the stage of the growth in thickness were used. For growing the plants, the wooden box (Fig. 1) to which a fine wire (p) with several wheels (q) at equal intervals is fixed just above and also which is provided with openings (o) at each side for sprinkling water (9).

Each of the plants was grown just under the wheel in a straight line, and on each of the wheels a string (Fig. 1, r) was hung and its one end was bound to a tip of the plant just under that wheel and the other end to a weight (Fig. 1, s) which is heavy enough to prevent the geotropic movement of the plant axis. By this means, it has been satisfactorily succeeded not only in keeping the axis in the horizontal or in a given inclined position, but in keeping it rightly in the horizontal position again, after it was rotated on its own axis by some given angles.

For the histological studies, the cross sections of the axis were taken from a midway portion of the hypocotyl as well as from that of the first inter-

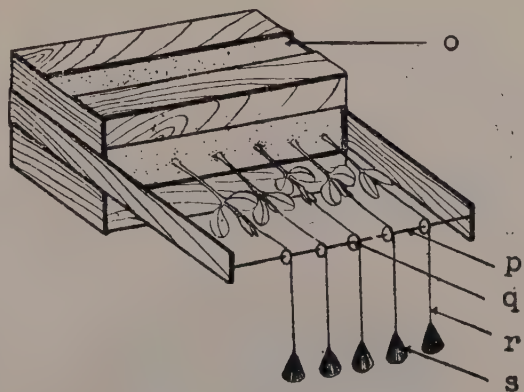


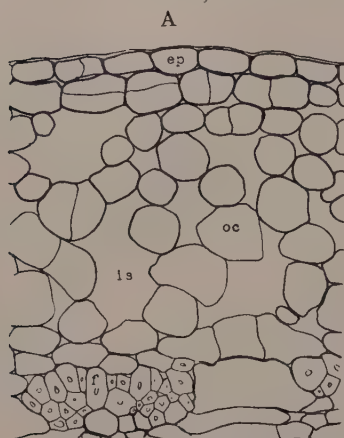
Fig. 1

node of the stem by free hand cutting. These were stained with the use of safranin and Delafield's haematoxylin combination and prepared for temporary mounts. In every experiment, 15-20 plants were used and the similar results were obtained, respectively.

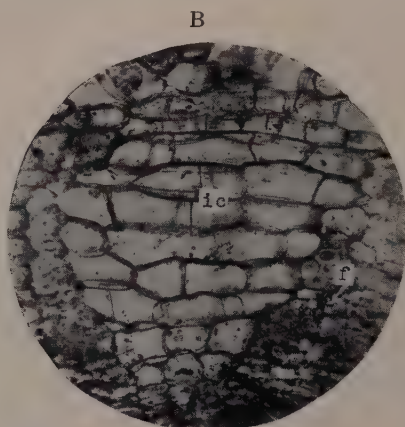
Results

1) Histological observations on the axes of jute plants

A) Observations on the vertical axes



× ca. 160



× ca. 60

Fig. 2.

The epidermis consists of 1 layer of cells (Fig. 2, A, ep) and the cortex more than 20 layers of cells. The outer portion of the cortex, viz. the portion between the subepidermis and the apical portion of phloem fibrous tissues, consists of 7-8 layers of round cells and shows large intercellular spaces among them (Fig. 2, A, oc and is), while the inner portion,



Fig. 3.

× ca. 17

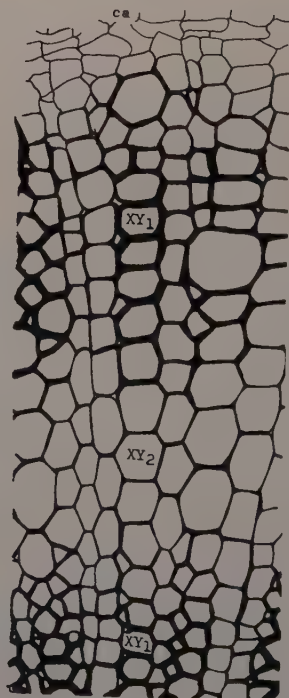


Fig. 4.

× ca. 160

viz. the portion between the phloem fibrous tissues is composed of some 10 layers of tangentially flattened cells which are densely laid one another (Fig. 2, B, ic).

The phloem fibrous tissues are formed around the periphery of the central cylinder with nearly equal intervals between themselves (Fig. 3, f). Each of them develops into a triangular shape in which 5-6 fibrous bundles are found formed. The cambial zone consists of 6-8 layers of cells.

In the xylem, the layers consisting of tangentially flattened, smaller cells with thick and well lignified walls (Fig. 3, XY₁ and Fig. 4, XY₁) and the layers of radially elongated, larger cells with thin and less or not lignified walls (Fig. 3, XY₂ and Fig. 4, XY₂) are arranged alternately with each other.

B) Observations on the horizontal axes and their comparison with the vertical ones

If the axis is horizontally kept, both the development of the inner portion of the cortex and the new differentiation of the xylem are remarkably accelerated. So that, its growth in thickness comes to be noticeable as a whole in contrast with that of the control vertically kept, and so far as the horizontal

axis is concerned, its growth in thickness is greater at the upper side than at the lower one, as the development of every tissue in the former (Fig. 5, U) is more conspicuous than in the latter (Fig. 5, L).

The outer portion of the cortex in both the upper and lower sides (Fig. 6, A and B, co) are similar to the control. However, the inner portion of the cortex is composed of 13-14 layers of tangentially flattened, larger cells in the upper side as against some 10 layers of smaller cells in the lower side, and as the cell divisions occur proliferously in both anticlinal and periclinal



Fig. 5.

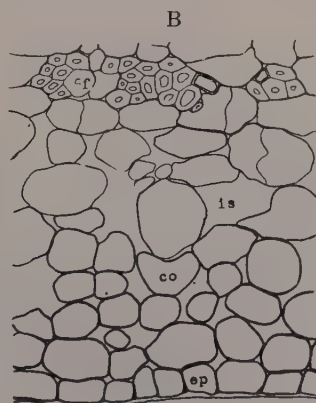
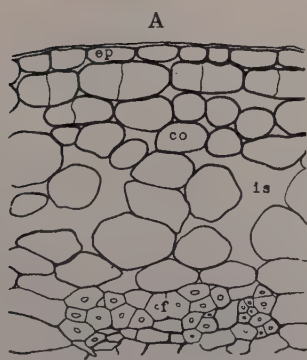


Fig. 6.

x ca. 160

directions in the inner portion of the cortex in the upper side, the tissue swells upward (Fig. 7, ic) and the outer portion of the cortex on that side (Fig. 7, oc) is pressed, becoming thinner.

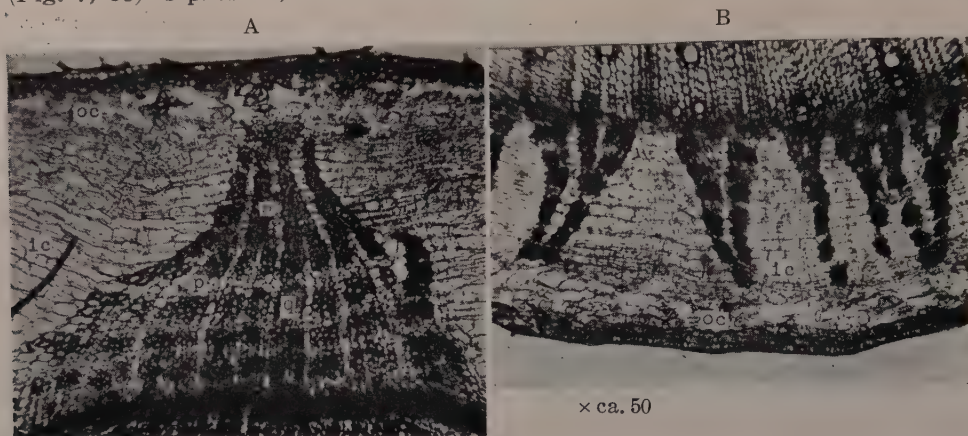


Fig. 7.

The formation of the phloem fibrous tissues in the upper side does not run parallel with such conspicuous development of the cortex as stated above, so that the boundary between the outer and inner portions of the cortex presents an undulation as shown in Fig. 5 and Fig. 7, A. While, in the lower side, the boundary becomes free from an undulation (Fig. 5 and Fig. 7, B).

The cell-layers in the cambium in the upper side are 6-8, being more numerous than those in the control, while those in the lower side are 4-5, being not so different from those in the control. Therefore, in the horizontal axis, the thickness of the cambial zone in the upper side exceeds that in the lower side.

A remarkable contrast is seen between the phloem fibrous tissues of the control (Fig. 3, f) and those of the horizontal axis (Fig. 5, f_U and f_L). In the horizontal axis the phloem fibrous tissues in the upper side are more accelerated than those in the lower side in formation, and so the former ones come to consist of a smaller number of the larger tissues sparsely laid (Fig. 5, f_U), while the latter ones consist of a larger number of the smaller tissues densely laid (Fig. 5, f_L). Phloem fibrous cells and parenchymatous cells formed in the upper side after the axis was horizontally fixed, become so much larger (Fig. 7A, p and q) than those which were already formed in that side before the axis was horizontally fixed (Fig. 7A, o) that in one and the same phloem fibrous tissue, the part formed before the treatment may be well distinguishable from the part formed after the treatment. In the lower side, such a remarkable change is hardly observed (Fig. 7, B).

The xylem formed in the upper side after the axis was horizontally fixed, presents a feature of the reaction wood which is generally composed of larger cells with thinner and less lignified walls and has a smaller number of vessels (Fig. 5, xy_U) compared with the normal xylem in the control (Fig. 3). Especially, 3-4 layers of cells formed immediately after the horizontal

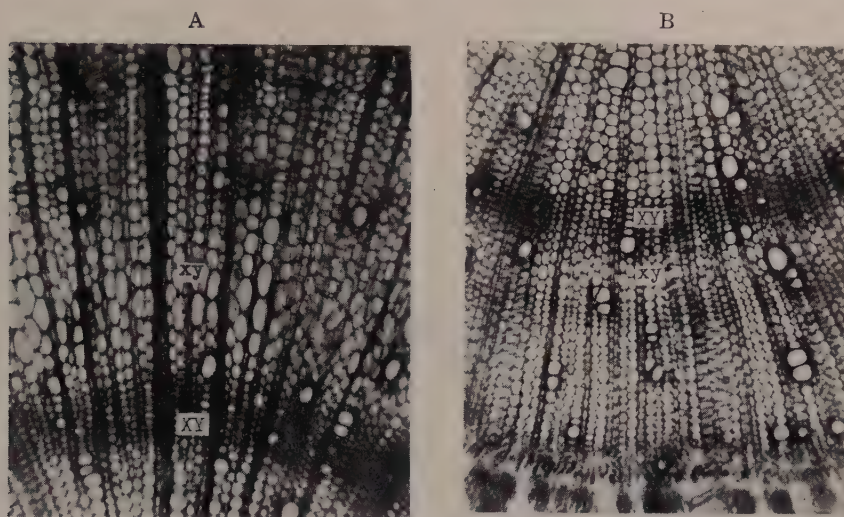


Fig. 8.

× ca. 35

treatment are remarkably elongated radially (Fig. 8A, xy), and then several layers of somewhat tangentially flattened, smaller cells are formed, but the formation of enlarged cells is occurred again up to just below the cambium (Fig. 5, xy_U). Whereas, the xylem formed in the lower side is generally composed of smaller cells with thicker and highly lignified walls (Fig. 5, xy_L), and although several layers of cells formed immediately after the horizontal treatment are somewhat thin-walled and elongated radially (Fig. 8B, xy), the thick-walled and tangentially flattened cells are again formed up to just below the cambium (Fig. 5, xy_L). By counting the number of cell-layers in the xylem formed in each side of one of the horizontal axes, it could be found that 22-25 layers in the upper side, 11-12 layers in the lower side and 15-20 layers in both the lateral sides are formed, respectively, after the axis was horizontally fixed.

**C) Histological changes shown
in the horizontal axis moved
by some given angles**

- a) A case in which the horizontal axis was rotated on its own axis by 90°.

The cortex and xylem become the thickest on the new upper side after the axis was rotated (Fig. 9, U₂), and those formed in the old upper side (Fig. 9, U₁) are more or less thicker than those formed

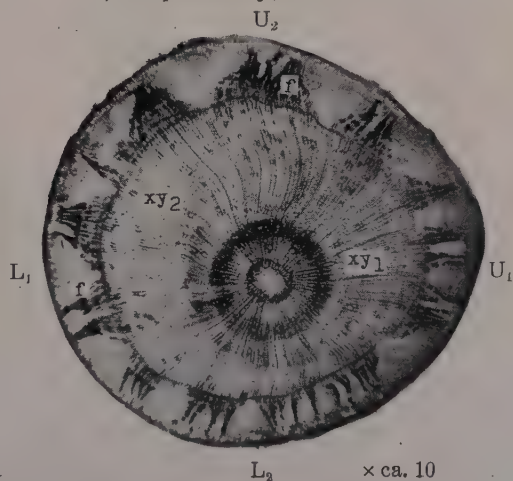


Fig. 9.

× ca. 10

in the opposite side, viz. the old lower

one (Fig. 9, L_1). Thus, every cross section of axes shows a contorted circle as illustrated in Fig. 9. In the xylem of the old upper side, the formation of reaction wood ceases (Fig. 9, xy_1 and Fig. 10, xy_1) and another new reaction wood is formed independently of the old one (Fig. 9, xy_2 and Fig. 10, xy_2). It is, however, a noteworthy fact that its formation takes place at the position extending from the new upper side to the new lower side in the opposite direction of the old reaction wood which now comes to be situated at one of the new lateral sides by the rotation.

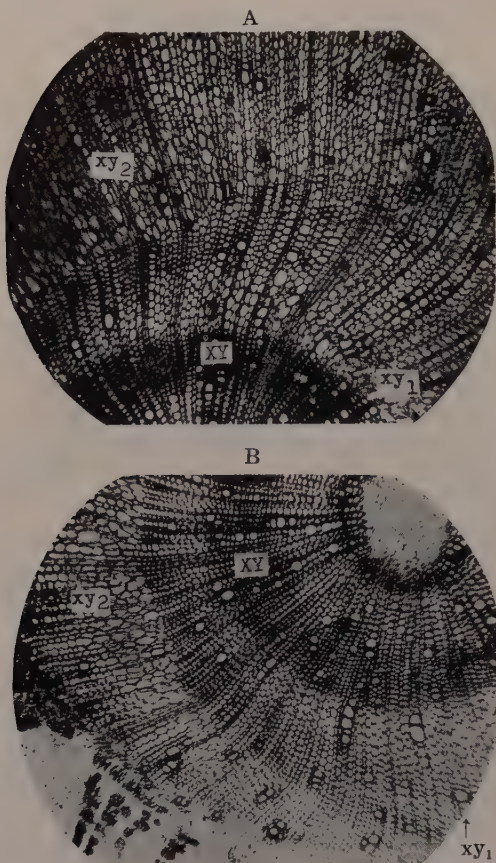


Fig. 10. \times ca. 40

of the horizontal axis already stated (Fig. 5, f_U).

b) A case in which the horizontal axis was rotated
on its own axis by 180° .

As the cell-divisions in the new upper side after the treatment of rotation are most accelerated, the inner portion of the cortex in that part thickens outward (Fig. 11, U_2). Whereas, in the new lower side, the proliferation as well as the growth of cells are somewhat retarded (Fig. 11, U_1) and thus, the cell-arrangements become similar to those in the upper and lower sides of the horizontal axis (Fig. 5, U and L), respectively.

In parallel with this, in the new upper side of the axis a smaller number of large phloem fibrous tissues composed of several rows of fibrous bundles are formed through the repeated division of cambial cells, while a larger number of small phloem fibrous tissues are formed in the new lower side, as the cambial activity is retarded in that side. Furthermore, a part of phloem fibrous tissues formed anew in the new upper side consists of thin-walled cells on the whole (Fig. 11, f_U) and parenchymatous cells in the phloem

fibrous tissues, especially those which were differentiated immediately after the treatment of rotation are strikingly elongated radially (Fig. 11, p). Thus, the differences between the part formed before and the part formed after the treatment of rotation are clearly distinguishable just like those seen in the phloem fibrous tissues in the upper side of the axis kept merely horizontally (Fig. 7, A). In the new lower side, the phloem fibrous cells formed during the first horizontal treatment are also thickened in their walls gradually; thus they come to be hardly distinguishable from those formed while the axis was vertically kept (Fig. 11, f_L).

The formation of the xylem is also accelerated on the new upper side (Fig. 11, xy₂), while on the new lower side, it is retarded (Fig. 11, xy₁), so that the cross section of that axis takes a long oval shape and the pith occupies the center. Moreover, as the cells of the reaction wood newly formed in the new upper side after the treatment of rotation have thin walls, elongate radially and become larger (Fig. 12, A, xy₂) than those already



Fig. 11.

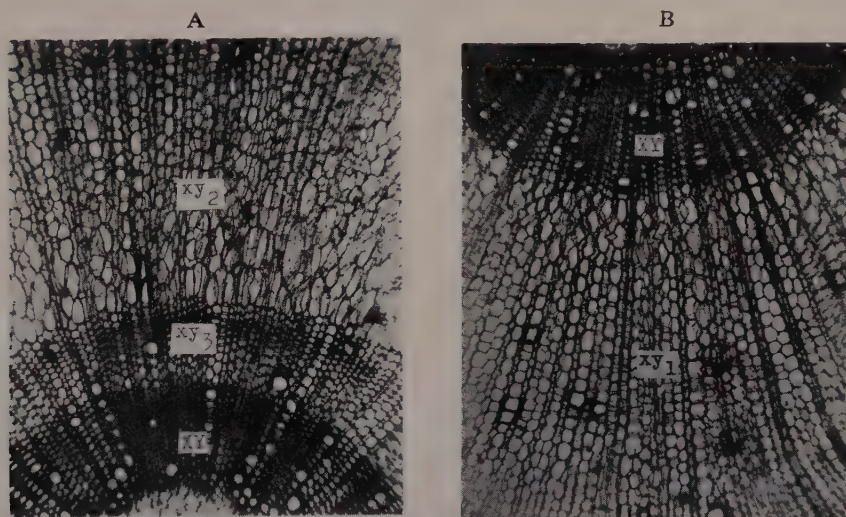


Fig. 12.

x ca. 55

formed in the upper side during the first horizontal treatment or in the new lower side after the treatment of rotation (Fig. 12, B, xy₁), they may be still more distinctly distinguishable from those already formed in the lower side during the first horizontal treatment (Fig. 12, A, xy₃). On the other hand, the walls of the cells already formed in the upper side during the first horizontal treatment, tend to thicken, gradually increasing in lignification,

c) A case in which the horizontal axis was turned to the vertical position.

If the horizontal axis is turned to and kept in the vertical position, every tissue comes to be formed evenly around the axis and thus, the cross section takes the shape of a circle (Fig. 13). The remarkable histological changes are as follows.

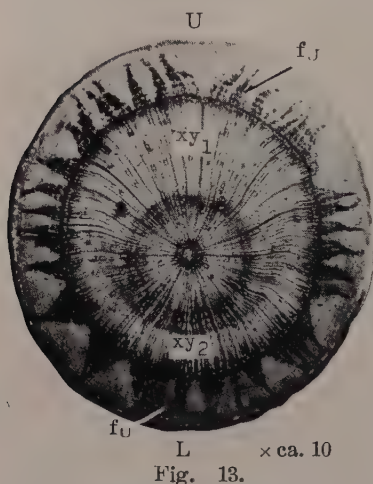


Fig. 13.

On the old upper side (Fig. 13, U), the increase in thickness of the cortex is retarded gradually, and on the old lower side (Fig. 13, L), it is accelerated. The development of phloem fibrous tissues runs also parallel with this change in the cortex. Namely, each of phloem fibrous tissues in the old upper side comes to be separated into several smaller ones (Fig. 13, f_U), while in the old lower side, several phloem fibrous tissues are each grouped into larger ones (Fig. 13, f_L). Thus, the phloem fibrous tissues around the axis begin to lose the respective features of size and shape seen in both sides through the first horizontal treatment (Fig. 5) and assume al-

most the same appearance as that shown in the vertical axis of the control (Fig. 3).

The formation of the xylem is also retarded and every xylem cell becomes smaller, increasing in lignification on the old upper side (Fig. 13, xy_1). Whereas, on the old lower side, the formation of the xylem is somewhat accelerated and every xylem cell becomes larger, thin-walled, decreasing in lignification (Fig. 13, xy_2). Thus, after the horizontal axis was brought to the vertical position, a quite similar tissue, though thin, to the reaction wood shown in the upper side of the ordinary horizontal axis comes to be formed in the xylem of the old lower side. However, it is only a temporary phenomenon and thus, the walls of the cells formed outside this thin reaction wood become gradually thicker and more highly lignified, returning to the similar condition shown in the xylem of the control vertically kept. Accordingly, the position of the pith is slightly shifted toward the new upper side and occupies near the center of the axis as compared with that of the mere horizontal axis.

2) Histological observations on the axes of hemp plants

A) The comparison between the vertical and horizontal axes

The histological structures shown in the cross sections of the axes of hemp plants vertically grown (Fig. 14) are very much the same as those of jute plants described above. In the axes of hemp plants, however, all over the insides of phloem fibrous cells, there are formed the solid cellulose layers which are not found in the jute plants. The axes of hemp plants horizontally kept, grow eccentrically in thickness just like those of jute plants. But, the

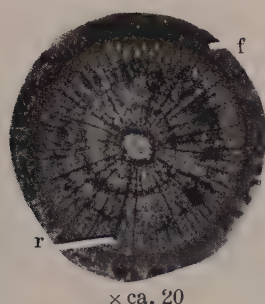


Fig. 14.

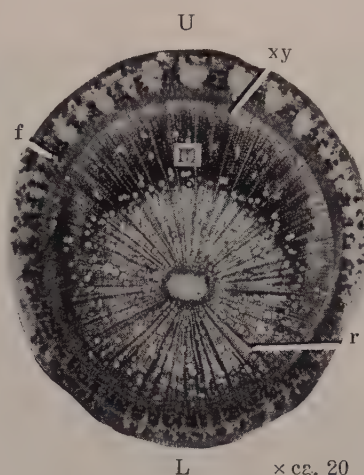


Fig. 15.

cells of the reaction wood, except the ray cells, form the mucilaginous layers all over their insides of secondary thickening membranes in the upper side (Fig. 15, m) and these

layers are of the same characteristics as those of the solid cellulose layers microchemically. Namely, they do not show any reaction to phlorogrucin and hydrochloric acid, but present a purple or brownish purple by the application of chlorzinc iodide, while they are in a great measure stretched with many folds and then dissolved by strong sulphuric acid. So, the layers may be assumed to contain a larger amount of some kind of cellulose or hemicellulose and a less amount of lignin.

Hitherto, it was considered that the mucilaginous layers are formed in the xylem cells of the reaction wood in the upper side of the inclined stems of arbors alone and not formed in those of herbs and shrubs. But the author has confirmed the fact that the axes of the plants which inherit the character of forming the solid cellulose layers by nature when standing upright, such as castor bean, *Ricinus communis*, flax, *Linum usitatissimum*, china grass, *Boehmeria nivea* and almost all of the leguminous plants, besides the hemp, *Cannabis sativa*, are generally able to form this layer in each cell of the reaction wood in the upper side, when they were horizontally kept or slightly inclined from the vertical position. And, from the fact that the mucilaginous layers found in a number of herbs coincide well with those found in arbors in regard to their conditions of formation, their forms and microchemical characteristics, etc., he suggests that these two are considered to be the same.

For the convenience of the comparison and contrast between the xylem in the upper side and that in the lower side of the same axis horizontally kept, a part of their tissues is illustrated in Fig. 16. The figures are arranged from the tissues formed just under the cambium to the tissues near the pith. It may be seen how conspicuous the formation of the xylem in the upper side (Fig. 16, A) is, compared with that in the lower side (Fig. 16, B). The extent of the xylem tissue from its inner part to the part marked with a dotted line in each side is the part already formed before the axis was horizontally kept. The size of every cell in these parts shows

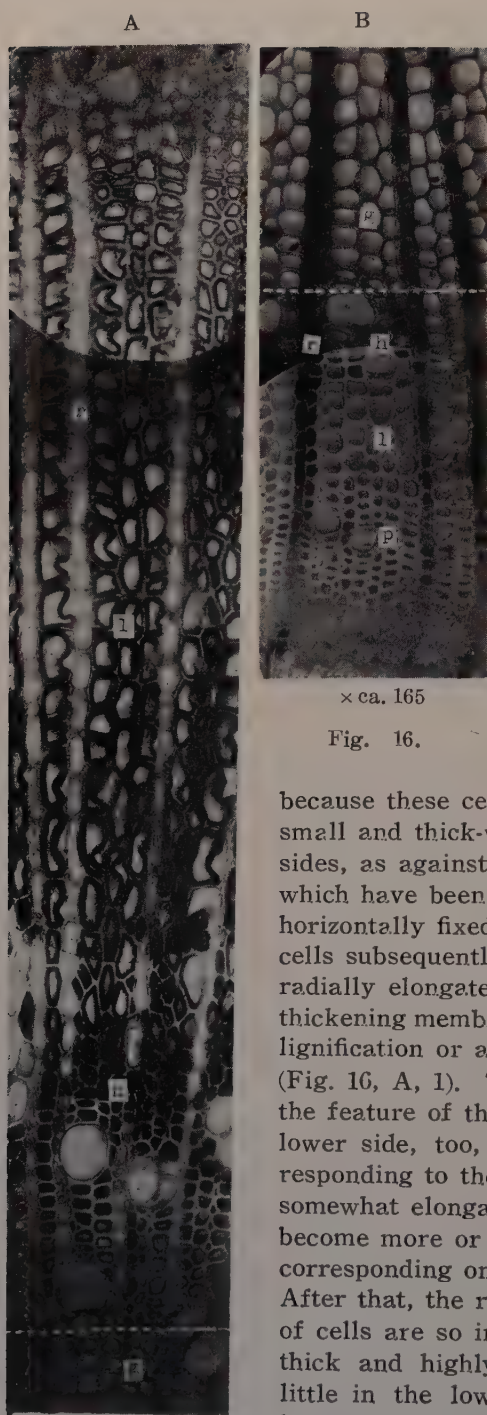


Fig. 16.

a remarkable dissimilarity between the upper and lower sides. This is, perhaps, due to the fact that the axis has ever happened to be slightly inclined before the experimental treatment was started. Judging from the aforesaid facts that the xylem cells in the horizontal axes of jute plants become larger at the upper side and smaller at the lower side, it may be well conceivable in this case also that the side composed of larger cells (Fig. 16, B, g) was situated at the upper side and the side of smaller cells (Fig. 16, A, g) at the lower side respectively, at that time.

In the axis of the hemp plant horizontally fixed, the xylem cells (Fig. 16, A and B, h) which were just differentiated from the cambium about the time when the horizontal treatment was made, seem to stop temporarily their growth all around the axis just after the treatment, because these cells covering several layers are very small and thick-walled in both the upper and lower sides, as against the cells occupying the inner part which have been already formed before the axis was horizontally fixed (Fig. 16, A and B, g). But the xylem cells subsequently differentiated in the upper side are radially elongated without thickening their secondary thickening membranes (ligneous walls), increasing their lignification or accelerating the formation of vessels (Fig. 16, A, l). Thus, they come to show apparently the feature of the reaction wood (Fig. 15, xy). In the lower side, too, several layers of xylem cells corresponding to the aforesaid ones in the upper side are somewhat elongated radially, but their ligneous walls become more or less thicker (Fig. 16, B, 1a) than the corresponding ones in the upper side (Fig. 16, A, l). After that, the rate of differentiation and enlargement of cells are so insignificant that the small cells with thick and highly lignified walls are formed only a little in the lower side (Fig. 16, B, p). Thus, in the horizontal axis, the xylem in the upper side amounts

to about 1.5 times as thick as that in the lower side as a whole (Fig. 15), and so far as the number of cell-layers formed after the horizontal treatment is concerned, the upper side reaches 3 times as great as the lower side.

While the xylem rays in the vertical axis are filled with a great quantity of starch grains (Fig. 14, r), those in the upper side of the axis horizontally kept contain none of them (Fig. 15 and Fig. 16, A, r) and those in the lower side in a great quantity (Fig. 16, B, r). Therefore, it can be said that the starch grains tend to disappear in the side of the axis showing the eccentric growth. Perhaps, it may be due to the fact that the starch grains are used up in the upper side for the active formation of cells and mucilaginous layers.

B) The formation of the mucilaginous layers shown in the horizontal axis moved by some given angles

a) A case in which the horizontal axis was turned to the vertical position.

If the horizontal axis (Fig. 15) is turned to and kept in the vertical position, every tissue, such as the cortex, xylem etc. becomes even around the axis in thickness, as its formation is accelerated on the old lower side and retarded on the old upper side. Thus, the cross section is of nearly a circular shape (Fig. 17). The phloem fibrous tissues are also accelerated on the old lower side (Fig. 17, f_L) and retarded on the old upper side (Fig. 17, f_U) in their formation, respectively. Thus, they come to show a similar appearance with each other.

In the xylem, while the formation of mucilaginous layers in the old upper side ceases (Fig. 17, m_1 and Fig. 18, A), it begins in the old lower side (Fig. 17, m_2 and Fig. 18, B, m_3). But, this is only a temporary phenomenon and in due course of time, the wood tissue without the mucilaginous layers comes to be formed all around the axis (Fig. 17, xy_1 and xy_2 . Fig. 18, A and B). The treatment of bringing the horizontal axis to the vertical position reveals also that the histological changes in the xylem, that is, the changes in the shapes and sizes of cells, the thickness of cell-walls and the formation of vessels, etc. are brought about. Namely, while the formation of vessels is accelerated, the cells grow smaller and their walls become thicker on the old upper side (Fig. 17, xy_1 and Fig. 18, A, xy_1), on the old lower side, the formation of vessels is somewhat retarded, the cells grow larger and their walls become thinner (Fig. 17, xy_2 and Fig. 18, B, xy_2) than those which were differentiated during the horizontal treatment (Fig. 18, B, XY). The starch grains disappear from the ray cells in the region where the mucilaginous layers are now formed in the old lower

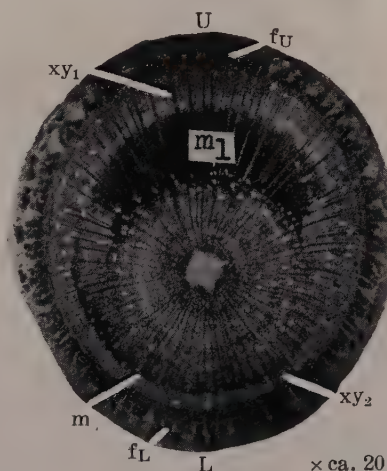


Fig. 17.

side (Fig. 18, B, r) and on the contrary, they appear in the ray cells of the newly formed region without mucilaginous layers in the old upper side (Fig. 17, xy₁ and Fig. 18, A, r).

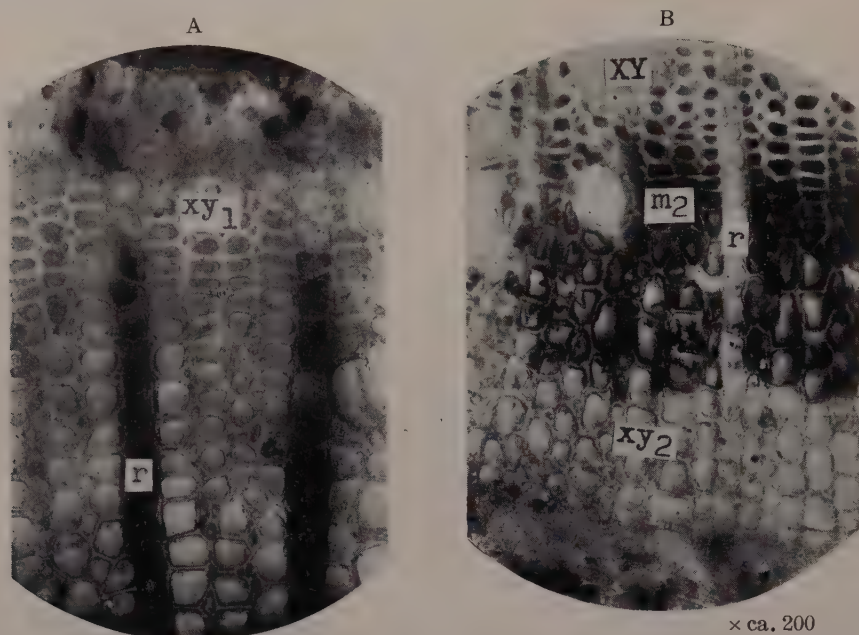


Fig. 18.

- b) A case in which the horizontal axis brought to the vertical position, was horizontally kept again by laying down in the same direction as it was before.

When the horizontal axis brought to a vertical position once is restored again to the horizontal position so that its upper and lower sides may keep the former position intact, the formation of every tissue, such as the cortex, phloem fibrous tissue, xylem, etc., is accelerated toward the upper side again. Thus, the cross section of the axis treated as such presents a long ovoidal shape and its pith is situated at the lower side eccentrically (Fig. 19). In the xylem of the upper side, there is formed anew another zone of mucilaginous layers outside the tissue which was formed during the treatment by which the horizontal axis was brought to the vertical position (Fig. 19, m₃). The innermost portion of this zone consists of several layers of very small, thick-walled and tangentially flattened cells ar-



Fig. 19.

ranged densely one another, and the remaining outer portion of this zone consists of large, thin-walled and radially elongated cells sparsely laid, as illustrated in the Fig. 16 A. In the lower side, there is formed the xylem tissue composed of small, tangentially flattened cells with thick and highly lignified walls, as illustrated in the Fig. 16 B, outside the thin tissue having the mucilaginous layers in its cells which have been formed while the horizontal axis was vertically kept (Fig. 19, m_3). Thus, it can be found that there are formed two zones of mucilaginous layers intervened by the ordinary xylem tissue in the upper side and one zone of mucilaginous layers between the ordinary xylem tissues in the lower side.

As already stated, the xylem rays are filled with starch grains with the exception of the part where the mucilaginous layers are formed.

- c) A case in which the horizontal axis brought to the vertical position, was horizontally kept again by laying down in the opposite direction.

When the horizontal axis brought to a vertical position once is restored again to a horizontal position, so as to be upside down, the formation of phloem fibrous tissues, xylem, etc. are accelerated on the new upper, viz. the old lower side (Fig. 10, U_2) and retarded on the new lower, viz. the old upper side (Fig. 20, U_1). Thus, the cross section of the axis become oval-shaped and the pith occupies the central part in the cross section.

Like the above-cited cases, the mucilaginous layers are formed in the xylem cells in the new upper side and the starch grains disappear from the ray cells in that part (Fig. 20, m_3 and Fig. 21, A, r) and in the new lower side the reverse phenomena take place (Fig. 20, xy and Fig. 21, B, r). Moreover, in the new upper side, the mucilaginous layers are formed in the cells which were differentiated during the treatment by which the horizontal axis was brought to a vertical position (Fig. 21, A, v_U) and accordingly correspond to ones illustrated in Fig. 17, xy_2 and Fig. 18, B, xy_2 . So that, in this case there can be seen two wide zones of mucilaginous layers which are situated at the opposite position each other in the xylem extending from the deep part to the part below the cambium (Fig. 20, m_2+m_3 and m_1).

Regarding the morphological and histological features shown in the reaction wood, there can be seen the following transition from the inner part toward the outer part by turns in both the upper and lower sides, respectively. In the new upper side, there are several layers of small cells with thick and highly lignified walls (Fig. 21, A, a_U) similar to those shown

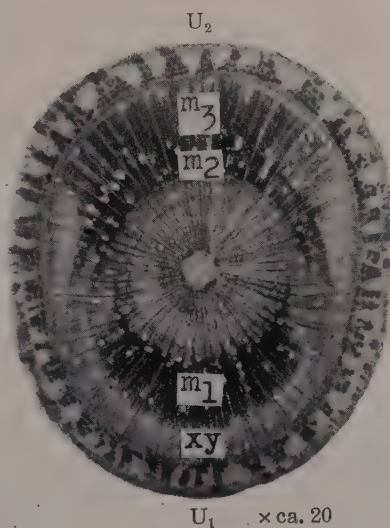


Fig. 20.

in Fig 18, B, XY as well as Fig. 16, B, p, and then several layers of somewhat enlarged cells with thick and fairly lignified walls (Fig. 21, A, v_U) similar to those in Fig. 18, B, xy_2 , with the exception that they have mucilaginous layers; further, next to them, a few layers of small cells with thick and highly lignified walls again (Fig. 21, A, b_U) similar to those illustrated in Fig. 16, A, h; then finally, a greater number of layers of radially elongated cells with thin and less lignified walls constituting the main part of the reaction wood as shown in Fig. 16 A, l (Fig. 21, A, l_U).

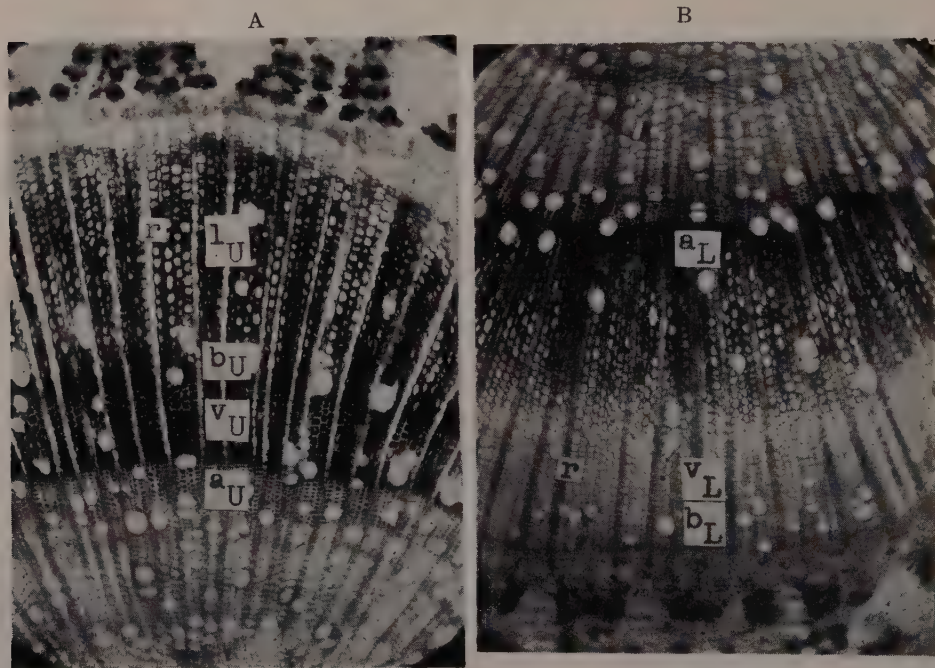


Fig. 21.

× ca. 60

Of these kinds of cell-layers, the former two are the tissues formed before the second horizontal treatment, though the mucilaginous layers in the cells of the outer portion of them are the ones formed in the new upper side during the second horizontal treatment and those in the cells of the inner portion of them are the ones formed while the horizontal axis was brought to a vertical position. In the new lower side, there are a few layers of small, thick-walled and highly lignified cells at the inner part and then a greater number of layers of tangentially elongated cells with thin and less lignified walls (Fig. 21, B, a_L). These two parts are quite similar to those illustrated in Fig. 16, A in their appearances and it is above suspicion that they were differentiated on the old upper side during the first horizontal treatment in view of the fact that the mucilaginous layers are formed here. The part adjacent to the zone of mucilaginous layers and composed of several layers of small cells with thick and fairly lignified walls (Fig. 21, B, v_L) resembles closely the part shown in Fig. 18, A, xy_1 and presents itself

that it was formed while the horizontal axis was vertically kept, since there is no trace of forming the mucilaginous layers. The outermost part which has many vessels and is composed of several layers of tangentially flattened and very small cells with thick and highly lignified walls (Fig. 21, B, b_L), is quite the same as that illustrated in Fig. 16 B, p. Of course, it is the part formed in the new lower side after the horizontal axis brought to the vertical position was fixed upside-down in the horizontal position again.

Consideration

It may be considered that, beside the effect of gravity, that of light, heat and humidity will also be different between the upper and lower sides, if the axis is horizontally fixed. But the similar phenomena to those shown in the results of the experiments were observed by keeping the axes of various plants horizontally in a dark room or by keeping horizontally their main roots which are now growing in the soil (Fig. 22). So that, it may be quite reasonable that the dissimilarity shown in the formation and structures of tissues between the upper and lower sides of one and the same axis should be mainly ascribed to the effect of change in the acting direction of gravity, and the effects of light, heat and humidity can be safely disregarded.

According to Priestley and Dorothy Tong, (8) in the horizontal woody stems of dicots, the wood formation is increased and the lignification retarded on the upper side, and the wood formation retarded and the lignification increased on the lower side. Onaka (6, 7) observed the fact that the mucilaginous layers are formed all over the insides of the xylem cells on the upper side of the horizontal woody stems of dicots.

Very little is known about such phenomena on the axes of herbs. But, the author confirmed by his materials that not only his results of experiments were well consistent with their observations, but the formation of the new reaction wood in the horizontal axis rotated on its own axis or in the horizontal axis brought to the vertical position took place in a remarkably different manner from that in the mere horizontal axis; namely, in the horizontal axis rotated by 90° , the new reaction wood was formed at the region near the lateral side in the opposite direction of the old reaction wood, in the horizontal axis rotated by 180° , it was formed in the old lower side, and in that brought to the vertical position, at the region in the old lower side, though thin and temporary in comparison with the former cases.

From these evidences, we know that the effect of changes of the angles between the main axis and gravity presents itself regularly and clearly in the form of histological changes. Accordingly, we can guess the relative

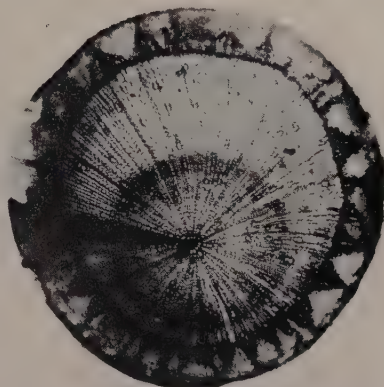


Fig. 22. \times ca. 12

changes in the past between the main axis and gravity, whether these changes might be made by natural or by artificial means, through the histological changes brought about in the tissues of main stems or main roots.

Moreover, it can be assumed that, whenever the direction of gravity upon a plant axis is changed, the transversal polarity is brought about in that axis, and on one hand, as the result of it, the substances, such as the plant growth hormones and enzymes which contribute to both the formative and metabolic activities of the cells come to change their distribution and the other conditions under the unilateral influence of gravity, and on the other hand, the formation of the new reaction wood goes through the influence of the accommodation of protoplasm which is caused by the preceding treatment and which maintains its effect for a time.

In regard to the distribution of plant growth hormones in the horizontal stems of needle-leaved trees, Onaka (7) has proved that the growth hormones are more abundantly distributed in the lower side than in the upper side and suggested that the fact will prove to be one of the causes to bring about the larger growth in thickness toward the lower side. The horizontal stems of broad-leaved trees grow larger in thickness toward the upper side than toward the lower side in like manner with jute and hemp plants, though he did not refer to its causes.

Now it is generally acknowledged that the plant growth hormones are allowing the plant axis to present the phenomenon of polarity by moving basipetally under the effect of gravity. In the horizontal axis, as the plant growth hormones may move from the upper side to the lower side and thus, accelerate the proliferation and growth of cells and the formation of tissues in the lower side, the phenomenon of hypotropy (the larger growth in thickness toward the lower side) may be well understood according to Onaka's suggestions. But the phenomenon of epitrophy (the larger growth in thickness toward the upper side) shown in the horizontal axes of jute and hemp plants or broad-leaved trees, is hardly understood by the polar movement of plant growth hormones alone. Such phenomenon seems to suggest that the way of response of plants to the plant growth hormones is different in every plant variety and tissue itself (14, 15).

For the purpose of making this point clear, the author is now engaged in making a research about the effects of plant growth hormones on the growth in thickness and the development of tissues, and though he does not yet arrive at a conclusion, he found the facts that the formation of the reaction wood on the upper side of the horizontal axis of the jute plant is retarded by the application of the heteroauxin-lanolin mixture, compared with that of the mere horizontal axis (Fig. 23) and moreover, the growth in thickness of the xylem in the vertical axis of an okra, *Abelmoschus esculentus*, which presents the same type of the growth in thickness with the jute plant when horizontally kept, is more retarded at the side applied by alpha-naphthaleneacetic acid than the opposite left untreated (Fig. 24). The further details of those histological descriptions apart, it may be inferred from the above statement alone that the epitrophical phenomena shown in the results

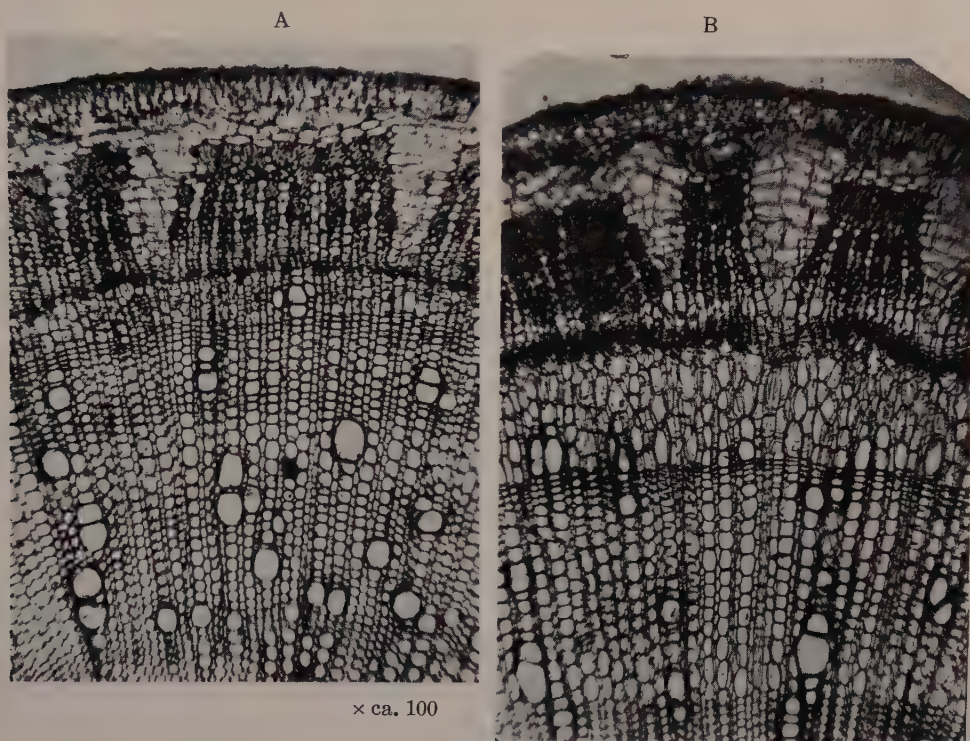
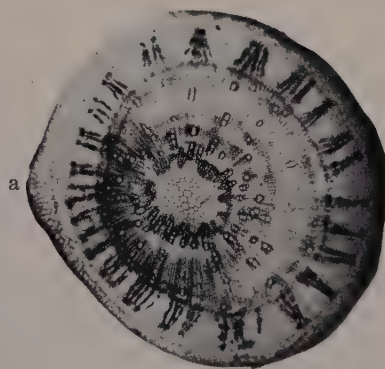


Fig. 23.

of the experiments have a close connection with the plant growth hormones. So that, the author wants to suggest tentatively a working hypothesis as follows: when the axis of a plant is horizontally kept, gravity acting along its transversal direction unilaterally comes to affect the distribution or the activity of a special kind of substances which have a connection with both the formative and metabolic actions, such as the plant growth hormones and enzymes, attending on the generation of the transversal polarity in the axis, and thus the differences of the growth in thickness and the development of tissues between the upper and lower sides are arisen.

Fig. 24. \times ca. 7

Résumé

1) If jute and hemp axes are horizontally fixed, they grow epitrophically, as almost all of their tissues are accelerated on the upper side and retarded on the lower side in their formation.

2) The lignification in both the phloem fibrous cells and xylem cells is retarded on the upper side and accelerated on the lower side. Thus, the xylem formed in the upper side shows the feature of the reaction wood, being composed of radially elongated, thin-walled cells sparsely arranged.

3) The xylem cells in the reaction wood on the upper side of the hemp axis horizontally fixed form the mucilaginous layers all over their insides with the exception of ray cells.

4) Besides the hemp plants, plants which form the solid cellulose layers in the phloem fibrous cells of the axes standing in the vertical position by nature, are generally able to form these layers, if the axes are moved from the vertical position and inclined even slightly.

5) The mucilaginous layers show a cellulose reaction to chlorzinc iodide, but do not show any reaction to phlorogrucin and hydrochloric acid. Thus, it may be considered that the layers contain a larger amount of some kind of cellulose or hemicellulose and a less amount of lignin.

6) The ray cells in the reaction wood of the lower side are generally filled with a great quantity of starch grains and those in the upper side contain none of them. This phenomenon is particularly noticeable on the plants whose axes are able to form the mucilaginous layers in their reaction wood on the upper side. Generally speaking, the starch grains tend to disappear in the side of the axis showing an eccentric growth. Perhaps, they may be used up for the active formation of cells and mucilaginous layers which take place in that side.

7) If the axis is kept inclined from the direction of gravity, that is, if the angles begin to exist between the main axis and gravity, its effect presents itself regularly and clearly in the form of histological changes. Accordingly, a clue for knowing whether the direction of the axis against gravity had ever changed or not in the past may be found through the close inquiry into the histological changes shown in the cross section of the axis.

8) The above-mentioned phenomena shown in the axis horizontally kept as well as in the horizontal axis moved by some given angles present themselves, independently of the effects of light, heat and humidity. So that, they may be chiefly due to the changes of the direction of gravity acting upon the plant.

9) The formation of the reaction wood in the horizontal axis of a jute plant is retarded by the application of the synthetic growth hormones and the growth in thickness of the vertical axis of an okra which grows epitrophically just like the jute axis when horizontally kept, is also retarded on the side applied with the same substances.

10) From the above-mentioned facts, it may be deduced that the transversal polarity is induced in the axis when it is moved from the vertical position and inclined, and subsequently gravity acting along the transversal direction of the axis affects the conditions of plant growth hormones and enzymes which are involved in the formative and metabolic activities of cells. Thus, the morphological differences are observed between the upper and lower sides of the axis.

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Explanation of figures

Fig. 1. Apparatus for fixing seedlings in the horizontal position.

o—opening, p—wire, q—wheel, r—string, s—weight.

Fig. 2. Cross section of cortex of a jute stem vertically kept.

A—outer portion, B—inner portion, ep—epidermis, f—phloem fibrous tissue, ic—cortical cells in the inner portion, is—intercellular spaces, oc—cortical cells in the outer portion.

Fig. 3. Cross section of a jute hypocotyl vertically kept, showing the appearance of phloem fibrous tissue and xylem.

f—phloem fibrous tissue, XY₁—xylem composed of tangentially flattened, smaller cells with thick and highly lignified walls, XY₂—xylem composed of radially elongated, larger cells with thin and less or not lignified walls.

Fig. 4. Cross section of xylem of a jute stem vertically kept.

ca—cambium, other abbreviations as in Fig. 3.

Fig. 5. Cross section of a jute hypocotyl horizontally kept, showing a remarkable eccentric growth.

U—upper side, L—lower side, f_U and f_L —phloem fibrous tissue, XY—xylem formed before the horizontal treatment, xy_U —xylem formed in the upper side after the horizontal treatment, xy_L —xylem formed in the lower side after the horizontal treatment.

Fig. 6. Cross section of the outer portion of a jute stem horizontally kept.

A—upper side, L—lower side, ep—epidermis, co—cortex, f—phloem fibrous tissue, is—intercellular spaces.

Fig. 7. Cross section of cortex of a jute hypocotyl horizontally kept, showing phloem fibrous tissue.

A—upper side, B—lower side, o—portion of the phloem fibrous tissue already formed before the horizontal treatment; cells are smaller and thick-walled, p—parenchymatous cells formed during the horizontal treatment; cells are remarkably enlarged, q—bundles of phloem fibrous cells formed during the horizontal treatment; cells are larger and thin-walled, ic—cortical cells in the inner portion, oc—cortical cells in the outer portion.

Fig. 8. Magnification of the portion of xylem illustrated in Fig. 5.

A—upper side, B—lower side, XY—cells formed before the horizontal treatment, xy—cells formed immediately after the horizontal treatment.

Fig. 9. Cross section of the horizontal hypocotyl of a jute plant rotated on its own axis by 90° .

U_1 —old upper side, U_2 —new upper side, L_1 —old lower side, L_2 —new lower side, f—phloem fibrous tissue, xy_1 —reaction wood formed before the treatment of rotation, xy—reaction wood formed after the treatment of rotation.

Fig. 10. Magnification of the portion of xylem illustrated in Fig. 9.

A—new upper side, B—new lower side, XY—cells formed before the first horizontal treatment, other abbreviations as in Fig. 9.

Fig. 11. Cross section of the horizontal hypocotyl of a jute plant rotated on its own axis by 180° .

U_1 —old upper side, U_2 —new upper side, f_U and f_L —phloem fibrous tissue, p—parenchymatous cells between the portions of the phloem fibrous tissue formed before and after the treatment of rotation, xy_1 —xylem formed in the old upper side before the treatment of rotation, xy_2 —reaction wood formed in the new upper side after the treatment of rotation.

Fig. 12. Magnification of the portion of xylem illustrated in Fig. 11.

A—new upper side, B—old upper side, XY—xylem already formed before the first horizontal treatment, xy_3 —reaction wood formed in the old lower side during the first horizontal treatment, other abbreviations as in Fig. 11.

Fig. 13. Cross section of the horizontal hypocotyl of a jute plant brought to the vertical position.

U—old upper side, L—old lower side, f_U and f_L —phloem fibrous tissue, xy_1 —xylem formed in the old upper side after the horizontal hypocotyl was brought to the vertical position, xy_2 —reaction wood formed in the old lower side after the horizontal hypocotyl was brought to the vertical position.

Fig. 14. Cross section of a hemp hypocotyl vertically kept.

f—phloem fibrous tissue, r—xylem ray.

Fig. 15. Cross section of a hemp hypocotyl horizontally kept.

U—upper side, L—lower side, f—phloem fibrous tissue, m—zone of mucilaginous layers, r—xylem ray, xy—reaction wood.

Fig. 16. Magnification of the portion of xylem illustrated in Fig. 15.

A—upper side, B—lower side, g—tissue formed before the horizontal treatment, h, l and p—tissues formed in sequence after the horizontal treatment, r—xylem ray.

Fig. 17. Cross section of the horizontal hypocotyl of a hemp plant brought to the vertical position.

U—old upper side, L—old lower side, f_U and f_L —phloem fibrous tissue, m_1 —zone of mucilaginous layers formed in the old upper side during the first horizontal treatment, m_2 —zone of mucilaginous layers formed in the old lower side immediately after the horizontal hypocotyl was brought to the vertical position, xy_1 —xylem formed in the old upper side after the horizontal hypocotyl was brought to the vertical position, xy_2 —xylem formed in the old lower side after the horizontal hypocotyl was brought to the vertical position.

Fig. 18. Magnification of xylem near the cambium illustrated in Fig. 17.

A—old upper-side, B—old lower side, r—xylem ray, XY—portion formed during the horizontal treatment, other abbreviations as in Fig. 17.

Fig. 19. Cross section of the horizontal hypocotyl of a hemp plant restored again to the horizontal position as it was, after it was brought to the vertical position.

U—upper side, L—lower side, m_1 —zone of mucilaginous layers formed in the upper side during the first horizontal treatment, m_2 —zone of mucilaginous layers formed in the lower side after the horizontal hypocotyl was brought to the vertical position, m_3 —zone of mucilaginous layers formed in the upper side during the second horizontal treatment.

Fig. 20. Cross section of the horizontal hypocotyl of a hemp plant restored to the horizontal position, so as to be upside-down, after it was brought to the vertical position.

U_1 —old upper side, U_2 —new upper side, m_1 —zone of mucilaginous layers formed in the old upper side during the first horizontal treatment, m_2 —complex zone of mucilaginous layers formed while the horizontal hypocotyl was brought to the vertical position (the inner portion) and those formed while it was restored to the horizontal position upside-down (the outer portion), m_3 —zone of mucilaginous layers formed in the new upper side during the second horizontal treatment, xy—xylem formed in the old upper side during the second horizontal treatment.

Fig. 21. Magnification of xylem near the cambium illustrated in Fig. 20.

A—new upper side, B—old upper side, au —tissue formed in the old lower side during the first horizontal treatment, bu and lu —tissues formed in the new upper side during the second horizontal treatment, vu —tissue formed in the old lower side after the horizontal axis was brought to the vertical position, al —tissue formed in the old upper side during the first horizontal treatment, bl —tissue formed in the new lower side during the second horizontal treatment, vl —tissue formed in the old upper side after the horizontal axis was brought to the vertical position, r—xylem ray.

Fig. 22. Cross section of the main root of a jute plant horizontally kept.

Fig. 23. Cross section of the portion of the horizontal stem of a jute plant, showing the effect of heteroauxin upon the development of a reaction wood on the upper side.

A—treated, B—untreated.

Fig. 24. Cross section of the vertical hypocotyl of an okra plant showing the effect of alpha-naphthaleneacetic acid upon the formation of tissues.

a—part treated.

Effects of the Vapour of Methyl 2,4-Dichlorophenoxyacetate on Growth and Differentiation in *Phaseolus vulgaris* L.

II. Behaviour of Decotylated Embryos in Germination *in vitro* and the rôle of Cotyledons in Formative Response.

By

Masaki FURUYA

(Received August 31, 1955)

Introduction

The first paper of this series (Furuya and Osaki, 1955) was devoted to the study of the formative responses induced in the seedlings after various grades of application with the vapour of methyl 2,4-dichlorophenoxyacetate on dormant bean seeds. In their work they found the malformation of first foliage leaves, the induction of unifoliolate leaf and the displacement of stipules and stipella in second foliage leaves, the formation of multifoliolate leaf in third and fourth foliage leaves, the change of phyllotaxis, and the anatomical modification of structural enlargement in transitional region between stem and root. Watson (1948) reported the relation between types of leaf injury and its location in *Phaseolus vulgaris* as a result of treatment with 2,4-D, and McIlrath and Ergle (1953, and 1953a) investigated the similar effects of 2,4-D in cotton plants. And also, Wilde (1951) reported anatomical modification of bean root-tips following soil-treatment with 2,4-D. These results showed that formative effects did not appear on the mature organ to which the chemical was applied directly, but occurred only on that formed in later. It is still, however, difficult to understand why gross and histological responses to these synthetic auxins may show a wide variety of patterns for a long duration of development.

The purpose of the present work is to find the answer to the question of how the vapour of methyl 2,4-dichlorophenoxyacetate applied to dormant bean seeds could stimulate and affect each organ to bring the above illustrated formative responses during the seedling.

Material and Methods

The strain, Master Piece, of bean (*Phaseolus vulgaris* L.) was used in all experiments. Air-dry seeds of this strain, harvested in the preceding autumn at Nagano Prefecture, were purchased from Sakata Seed Co. Ltd., and the material used in present work was carefully selected in order to avoid those which were damaged or either extremely big or small.

Treatment with the vapour of methyl 2,4-dichlorophenoxyacetate.—Dormant dry intact seeds were exposed for 5 weeks in Mullison and Hummer (1949)'s container saturated by the vapour of the chemical and placed in a dark room under constant temperature at 25°C, as in previous work (Furuya and Osaki, 1955).

In application to dormant decotylated embryos, all experimental procedures were made in aseptic condition, providing for succeeding sterile cultures. 50-ml. Erlenmayer flask with a stopper was used as storage container for exposing decotylated embryos to the vapour of the chemical, and a sufficient amount of the chemical was placed in the bottom of each flask. Dormant decotylated embryos were excised aseptically from untreated air-dry seeds with the aid of a scalpel. Small gauze bag containing twenty decotylated embryos was suspended from the eye-bolt of the stopper, and then the stopper of container was sealed up with paraffine. The containers were placed under the above described condition for several long hours.

Sterile culture technique of decotylated seedlings.—Cotyledons were removed from dormant air-dry seeds or from growing young seedlings: in the former case, excision of cotyledons, either pretreated with the chemical or not, was done just after the sterilization of the surface of seed-coat by the illumination of ultra-violet lamp for about 10 minutes; in the latter case, the intact air-dry seeds were surface-sterilized in 0.15% Uspulun solution for 20 minutes, rinsed in sterile water, and germinated upon the moist sand in the dark at 25°C until the removal of cotyledons was carried out with sterile techniques. Then, just after the operation of excision, decotylated embryos, or seedlings, were transferred to a 50-ml. Erlenmayer flask containing 15-ml. of a basic mineral nutrient medium to which 1 per cent agar and 0.5 per cent sucrose had been added. The basic mineral nutrient medium contained in mgr./l. of distilled water: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 50; KNO_3 , 50; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50; K_2HPO_4 , 20; KCl , 20; $\text{Fe}_2(\text{SO}_4)_3$, 0.2; H_3BO_3 , 0.1; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.1; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; KI , 0.1. Medium was adjusted to 5.8-pH, and then autoclaved at 15 pounds pressure for 10 minutes after all constituents had been added.

For several days after germination, the cultures were maintained at 25°C in the dark condition, and after that, transferred to the chamber lightened continuously from above by four 20-watt Mazda fluorescent tubes under the temperature kept at 24-28°C. They were maintained there throughout the seedling stage. No control of humidity was attempted.

Results and Discussion

In previous work (Furuya and Osaki, 1955), it was noted that both gross and histological responses of seedlings showed a wide variety of patterns as a result of treatment with methyl 2,4-dichlorophenoxyacetate. Here, some interpretations might be offered for this phenomenon. That is, if the embryonic tissues of dormant bean seeds are directly affected by the chemical, such a wide range of variety in response will result from the difference

in age of each organ, or tissue, at the time of treatment with the chemical. Contrariwise, if the vapour of the chemical do not stimulate directly to embryo enveloped by seedcoat and cotyledons, the cotyledons will work as the source of a secondary stimulus to induce several modifications in the process of organ-formation. In short, here are two alternative hypotheses: (1) whole parts of seeds, including the embryonic organs, are stimulated by the chemical for the duration of treatment, or (2) only the outer part, i.e. cotyledon, is affected. To resolve experimentally the question of whether hypothesis is possible, a series of experiments were made as follows.

1. **Growth and behaviour of decotylated embryos excised from pre-treated dormant seeds.** The present project was undertaken in an attempt to determine whether the chemical has penetrated into the embryonic parts of dormant seed for the duration of application. Two experimental groups of dormant decotylated embryo, one of which was excised from resting air-dry seeds that exposed to the vapour of methyl 2,4-dichlorophenoxyacetate for 5 weeks and another of which excised from untreated control beans, were set up. These decotylated dormant embryos were planted aseptically in an erect position on the surface of the nutrient medium, and grew in the dark condition above mentioned for 4 days after inoculation. After that, these cultures were transferred to the lightened room and maintained there for about 2 months until the end of these development. Rate of germination, growth rate of whole plant and of main root, morphological characters of seedling were studied under various stages of growth.

All decotylated embryos have germinated *in vitro* in both groups, as to be expected from the result in author's previous work (1955). The pretreated decotylated seedlings have grown as rapidly as untreated controls did, and within the age of 4-days, some of seedlings began to form their lateral roots in both groups as shown in figure 1. Any difference of growth and of behaviour between both groups could not be noticed for this dark period.

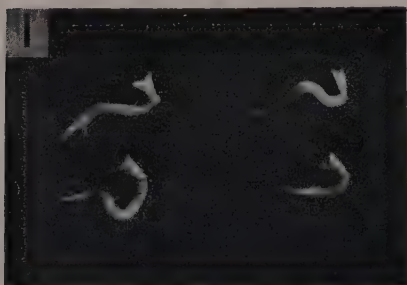


Fig. 1. Decotylated seedlings, grown *in vitro* after a 4-days growth period. Left, seedlings excised from pretreated bean seeds; right, ones from untreated controls.

After that, both groups of the etiolated decotylated seedlings were transferred to the lightened room. Then, they began to produce chlorophyll in the tissues of first foliage leaves and of upper hypocotyls. After a 10-days growth period, the blades of first unifoliolate leaves, in which chlorophyll has developed thoroughly, were almost expanded (fig. 2) in the greater part of the number of plants in both groups, and in a few, fully expanded in normal form as demonstrated in figure 3.

At the time of 16-days old, the fresh weight of decotylated seedlings and the length of main root were measured in order to examine how the pre-

treated group differs from the untreated control. For all the variables measured, mean values and standard errors were calculated. This result was given in table 1. These data show that the difference of growth rates

Table 1. Comparison of growth rates between decotylated seedlings excised from dormant seeds pretreated with the vapour of methyl 2,4-dichlorophenoxyacetate and those from the untreated controls. Measured at the age of 16-days.

Treatment to seeds from which decotylated embryos were excised	Fresh weight of decotylated seedling		Length of main root	
	Number of samples	Mean and standard error (mgr.)	Number of samples	Mean and standard error (mm.)
TREATED	20	98.0 ± 12.9	15	23.9 ± 5.5
UNTREATED	20	90.0 ± 13.9	14	24.9 ± 3.8
Analysis of variance	F=3.36 < F _{0.05} =4.10		F=0.296 < F _{0.05} =4.21	

between both groups is not significant at the five per cent point according to the analysis of variance (Snedecor, 1948), as stated in table 1.

In due course of time, first, second, third, and fourth trifoliate foliage leaves developed successively into normal form (fig. 4) in both groups of cultures, main and lateral roots also grew well, and all plants *in vitro* already died within 2 months after inoculation. Any formative effect was

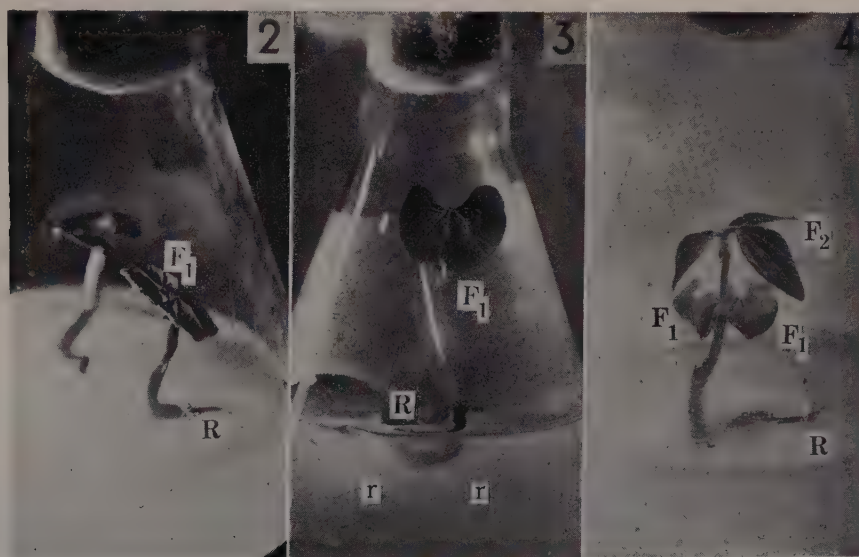


Fig. 2. Decotylated seedling in the time of 10-days old, excised from control seed. F₁, first foliage leaf; R, main root.—Fig. 3. That (10-days old) from pretreated seed, showing no modification in form. r, lateral root.—Fig. 4. Second foliage leaved stage of pretreated decotylated seedling which represented no formative response. Growth period, 17-days. F₂, second foliage leaf.

not appreciable throughout the development of seedlings except reduction of size in comparison with the intact normal plants.

To test whether the seeds employed had been sufficiently exposed or not to the vapour of methyl 2,4-dichlorophenoxyacetate, in each case of present experiments, some of treated seeds were sown in pots filled with a mixture of sand, loam, and humus in greenhouse, and respective formative responses as the result of perfect application with the chemical were confirmed.

The data presented here give evidence that the decotylated embryos have not been affected yet by the stimulus of methyl 2,4-dichlorophenoxyacetate, but it is still difficult from only above results to conclude that the chemical did not penetrate into the tissues of embryos other than cotyledons for duration of treatment.

Vyvyan (1924) showed the effect of removal of cotyledons upon reduction in the rate of growth of first foliage leaves in bean plants. Rippel (1937) found that the cotyledons are necessary for normal lateral root initiation in the intact pea seedling, and Torrey (1952) studied that the shoot plays unmeasurable rôle in lateral root initiation whether or not the cotyledons are present, and that by the treatment, in which the cotyledons were substituted by the lanoline paste mixtures of IAA, there was no effect on lateral root formation. Therefore, it was thought possible that the cotyledons may play important rôle for normal ontogeny of bean seedling whether the chemical was applied or not.

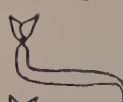





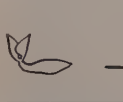
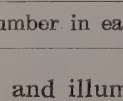
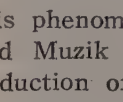
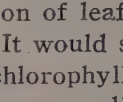
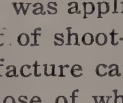
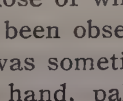
2. Application with the vapour of methyl 2,4-dichlorophenoxyacetate directly to decotylated dormant embryos. This becomes a next subject to study what behaviours will be observed in the development of decotylated embryos exposed directly to the vapour of methyl 2,4-dichlorophenoxyacetate for several long hours at the dormant stage.

The dormant decotylated embryos, excised aseptically from dry untreated bean seeds, were exposed in the space saturated by the vapour of the chemical for 0, 1, 2, 4, 10, and 32 days respectively. After such application of the chemical, the decotylated embryos were planted in nutrient media and cultured in dark condition for 1 week, then transferred to lightened condition until these seedlings died. Thus, directly treated embryos have behaved in many way as a result of the different duration of treatment, and the data obtained from this experiment are summarized in tables 2 and 3.

It is interest that twelve patterns of formative effects given in table 2 had nothing in common with those previously obtained in the intact bean plants (Furuya and Osaki, 1955), and that the degree of inhibition was related to the duration of exposure to the chemical in present work, whereas, in previous work, this tendency was not seen beyond a threshold of the exposure-period and the frequency with which these responses occurred was found at constant level. And the depression of germination was observed in decotylated embryos directly treated for 4 or more weeks, but any influence could not be found in the rate of germination in pretreated intact seedlings.

Decotylated embryos, treated directly for 4 days or more, did not produce chlorophyll at all, in spite of the existence of a necessary amount of

Table 2. The relationship between duration of exposure to the vapour of methyl 2,4-dichlorophenoxyacetate and behaviour of decotylated embryos in sterile cultures *in vitro*. Data are shown by the number of plants representing each pattern of development.

Pattern of development	Feature of seedling (one week old) → (five weeks old)	Duration of exposure (days)						Remark
		0	1	2	4	10	32	
I		20	4	1				
II			2	1				
III			6	2				
IV			1					
V			7	2	8			see Fig. 6
VI				5				see Fig. 12
VII				2		4		see Fig. 9
VIII				4	2			
IX				2	7			
X						14		see Fig. 11
XI						2	4	see Fig. 10
XII							16	
Total number in each experimental series		20	20	19	17	20	20	

minerals and illumination for the production of the green pigment. Therefore, this phenomenon differs from so-called chlorosis or etiolation. Loustalot and Muzik (1953) found that the velvet bean seedlings showed a sharp reduction or cessation of photosynthesis and extensive damage and destruction of leaf mesophyll after treatment with 2,4-D applied as a spray or dip. It would seem that synthetic auxins may affect generally to formation of chlorophyll, or photosynthesis, when relatively large amount of the chemical was applied. In such case, no organ could develop from the growing point of shoot-apex, and such pale yellow plants are probably unable to manufacture carbohydrate because of this absence of chlorophyll.

In those of which shoots developed, no gross and histological modification has been observed in stem- and leaf-formation, though a retardation of growth was sometimes exhibited as in the patterns II to IV in table 2. On the other hand, pale yellow plants representing the patterns V to XII have

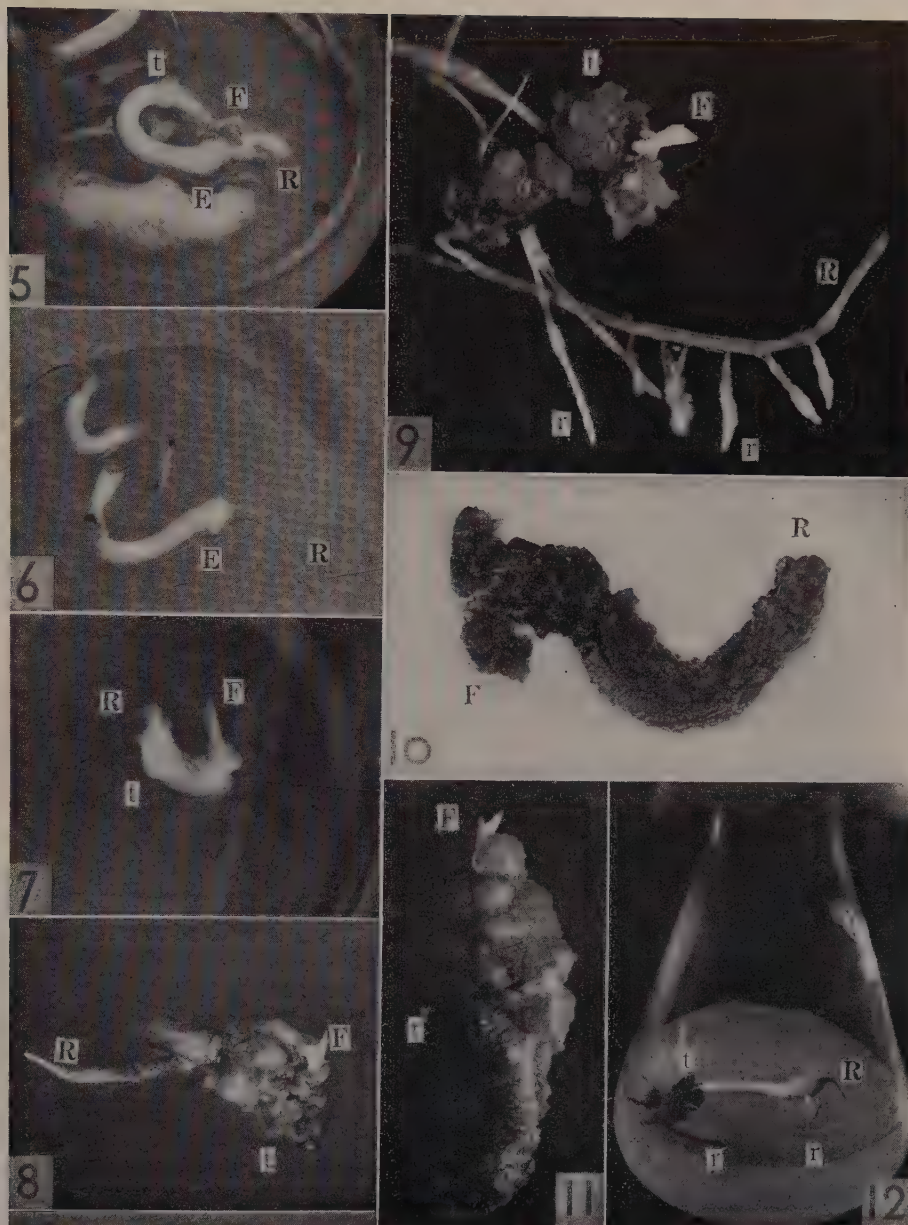
Table 3. Symptom in twelve patterns given in table 2. (+, occurred distinctly; \pm , occurred slightly; —, for no occurrence)

Characteristics of decotylated seedlings	Pattern of development (same number as employed in table 2)											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
OBSERVED IN THE AGE OF 1 WEEK												
a) elongation of hypocotyl	+	+	+	—	+	+	\pm	\pm	\pm	\pm	—	—
b) growth of main root	+	\pm	\pm	—	\pm	\pm	\pm	—	—	—	—	—
c) initiation of lateral roots	\pm	—	—	—	—	—	—	—	—	—	—	—
d) swelling in region between stem and root	—	—	\pm	—	+	\pm	\pm	+	+	+	+	—
e) tumorization in tissue near cotyledonary node	—	—	—	—	—	\pm	\pm	\pm	+	+	+	—
OBSERVED IN THE AGE OF 5 WEEKS												
a) formation of successive foliage leaves	+	+	+	\pm	—	—	—	—	—	—	—	—
b) production of chlorophyll in leaves and upper hypocotyl	+	+	+	+	—	—	—	—	—	—	—	—
c) growth of hypocotyl	+	+	+	\pm	+	+	+	\pm	\pm	+	—	—
d) internodal growth of epicotyl and upper stem	+	\pm	\pm	—	—	—	—	—	—	—	—	—
e) growth of main root	+	+	+	—	\pm	\pm	\pm	—	—	—	—	—
f) formation of lateral root on main root	+	+	\pm	—	—	\pm	\pm	—	—	—	—	—
on transitional region between stem and root	+	—	—	—	—	+	+	—	—	—	—	—
on hypocotyl	—	—	—	—	—	+	+	+	—	—	—	—
on mid-rib of first foliage leaves	—	—	—	—	—	+	+	—	—	—	—	—
g) conspicuous structural enlargement between stem and root	—	—	+	—	+	+	\pm	+	+	—	—	—
h) proliferation of tumorous tissue from main root	—	—	—	—	—	—	—	—	—	\pm	+	—
from transition region between stem and root	—	—	—	—	—	—	+	—	—	+	+	—
from upper hypocotyl	—	—	—	—	—	+	+	\pm	+	+	+	—
from ungrown first foliage leaves	—	—	—	—	—	\pm	\pm	—	\pm	\pm	+	—

not demonstrated any normal growth and function in shoot-development, and have often formed lateral roots from the veiny portion of the pale, not growing first foliage leaves (fig. 12), and proliferated tumorous tissue enormously (figs. 9, 10 and 11).

It was generally observed in this experiment that the growth of radicle has been prevented irrespective of the duration of treatment to decotylated embryos. But, when stimulated by exposure to the chemical for 2 to 4 days, many lateral roots have been induced markedly from all portion of embryo, i. e. first foliage leaves, hypocotyl, transitional region between stem and root, and main root.

It would appear that only the conspicuous structural enlargements in the region between stem and root could occur by direct stimulation with the chemical, though all of the other formative responses formed in plants with



Behaviour *in vitro* of decotylated bean embryos pretreated directly with methyl 2,4-dichlorophenoxyacetate at dormant stage. R, root; F, first foliage leaf. Figs. 5 and 7. Seedlings pretreated for 48 hrs. showed abnormal structural enlargement in region between stem and toot (E) and tumorous proliferation (t) in upper hypocotyl, at the age of 8th day.—Fig. 6. That pretreated for 24 hrs., aging in 8 days old.—Fig. 8. Explant pretreated for 4 days, in a 40 days growth period.—Figs. 9 and 12. Cultures, pretreated for 48 hrs, showed well developping lateral roots (r) and tumorous masses, in the age of 20 days.—Figs. 10 and 11. Embryos pretreated for 10 days developed to a buld of tumor-masses, 30th day growth period.

FURUYA: Effects of 2,4-D-methyl ester on growth and differentiation in *Phaseolus*, II.

cotyledons could not be induced. This fact was expected from the early works that conspicuous shoulders of tissue in the back of the tip in roots had been produced by the soil-treatment with 2,4-D (Wilde, 1951) and from other example of similar phenomenon described by Boas (1949) as a result of treatment with eosin. Also, cultures *in vitro* of decotylated bean embryos could induced such shoulders as a result of treatments with several auxins (Furuya and Soma, in press). However, such structural enlargement was not induced in the decotylated seedlings excised from dormant seeds pretreated with methyl 2,4-dichlorophenoxyacetate, while the previous paper represented that such modification was produced in pretreated intact seedlings. Therefore, this conspicuous shoulder of tissue may be induced by both the direct stimulus to the embryo and the indirect stimulus through cotyledons. When the shoulders were induced on decotylated seedlings in culture *in vitro*, these were reduced in size as compared with those produced on intact plants.

In a number of decotylated seedlings, pretreated directly for 2 days or more, extensive cell proliferation began in the region near by cotyledonary node within a week after germination (figs. 5 and 7). Then, the tumourization progressed to upper hypocotyl and sometimes developed to first foliage leaves and/or to lower portion of hypocotyl and root. These *callus*-like masses have slowly grown for several months (fig. 10), and in the patterns VI and VII, lateral roots have initiated from every parts of proliferated tissues in the later period of development.

Thimann (1951) pointed out that, in general, alkyl esters of the acid-auxins are active, and methyl ester has about the same activity as the acid. Such fact ascertained by the present work might be demonstrate in comparison with Loustalot and Muzik's experiment (1953): the patterns II to IV in table 2 are analogous to the case of the application with 0.001% 2,4-D, the patterns VI, VII or VIII coincide with that in the treatment with 0.01% 2,4-D, and the pattern XII is equal to 0.1% 2,4-D application.

Addenda: When the decotylated embryos were excised aseptically from untreated dormant seeds and transferred to a Erlenmayer flask containing nutrient medium, saturated with the vapour of methyl 2,4-dichlorophenoxyacetate, they might grow normally for a short time in the beginning of germination, and soon after, began to proliferate tumorous tissues, and still later, ceased developing either in the dark condition (Fig. 13) or in the light (Fig. 14).

3. Relationship between induction of formative responses and duration of maintenance of cotyledons in pretreated bean seedlings. If the embryonic parts of intact seed had absorbed, more



Fig. 13. Decotylated seedling, grown in the space saturated by the vapour of methyl 2,4-dichlorophenoxyacetate under the dark condition.—Fig. 14. Under the light condition.

or less, the vapour of methyl 2,4-dichlorophenoxyacetate for the duration of treatment, the embryos excised from treated seeds would have showed some pattern of formative response cited in table 3 in proportion to the amount of the chemical absorbed. However, the data presented in chapter 1 demonstrated that the explants excised from treated seeds did not show any formative response in their development. In consequence, the following conclusions may be drawn: (a) when intact bean seeds were exposed to the vapour of methyl 2,4-dichlorophenoxyacetate, no or a few amount of the chemical might penetrate into the tissue of embryos; (b) growth and differentiation of decotylated embryos treated directly by the vapour of the chemical for different duration showed a wide variety of pattern, but differed from those of intact seedling as studied in previous work. Thus, in view of the fact that such a rôle of cotyledon in induction of formative effects has been established, the possibility of hypothesis 1 (*see* page 272) is minimized. Now, the contrary possibility, i. e. hypothesis 2, must be investigated in details in following chapters.

It is evident that the cotyledons are necessary to occur such formative effects as in the previous paper (Furuya and Osaki, 1955). An attempt was made to resolve the question of how long the cotyledon must be maintained in order to bring such formative effects on growing seedling. That is, both cotyledons were removed from seedling at various developmental stages, and then, the decotylated seedlings were planted aseptically on nutrient medium. In this experiment, when the removal of cotyledons was done within 3 days old, the decotylated seedlings were cultured *in vitro* aseptically as above mentioned, but the others decotylated in later were grown by use of water-culture method. In the fifth day after germination, the seedlings, whether or not the cotyledons have been removed, were transferred from a dark room to a continuously lightened room, and maintained there until they died. The data obtained here are given in table 4.

It can be concluded that each formative response has required different duration of maintenance of cotyledons respectively, and that the induction of such modification has ceased soon after the cotyledons had been removed. In general, the later the cotyledons were excised from seedlings in course of germination, the more severe the modification of decotylated seedlings became in sterile cultures. Elongation of main roots and formation of lateral roots were found more in early dark period than in the later light period. Torrey (1952) reported that white light apparently inactivated the substances moving from the cotyledons and being essential for lateral root formation.

It was thought that a growth regulating substance, or substances, moving from treated cotyledons plays important rôle to induce such formative effects. However, it is still unknown whether this substance is methyl 2,4-dichlorophenoxyacetate itself which has been applied to the seeds, or this is a secondary stimulus produced in the cotyledons affected by treatment with the chemical. Kögl and Kostermans (1935) found in the *Avena* test that activity decreased with the increasing size of the alkyl esterifying group and concluded that the esters must be hydrolysed to the free acids to

Table 4. Relation between occurrence of formative responses in seedlings and duration of maintenance of cotyledons pretreated with methyl 2,4-dichlorophenoxyacetate. +, occurred in all plants; \pm , occurred in some plants, but not in the others; —, not occurred in any plant.

Sympton of formative responses	Application with the chemical to seeds	Duration of maintenance of cotyledons in the seedlings						
		(Hours)				(Days)		
		3	24	48	72	5	7	10
Inhibition of growth in main root	treated	—	\pm	+	+	+	+	+
	untreated	—	—	—	—	—	—	—
Formation of structural enlargement in region between stem and root	treated	—	—	\pm	+	+	+	+
	untreated	—	—	—	—	—	—	—
Delay in development	treated	—	—	\pm	\pm	+	+	+
	untreated	—	—	—	—	—	—	—
Gross and histological modification in first foliage leaves	treated	—	—	—	—	\pm	+	+
	untreated	—	—	—	—	—	—	—
Induction of unifoliate leaf in second foliage leaves	treated	—	—	—	—	\pm	\pm	+
	untreated	—	—	—	—	—	—	—
Displacement of stipules and stipella in second foliage leaf	treated	—	—	—	—	\pm	\pm	+
	untreated	—	—	—	—	—	—	—
Formation of multifoliate leaf in 3rd and 4th foliage leaves	treated	—	—	\pm	—	—	\pm	+
	untreated	—	—	—	—	—	—	—

produce growth, and Thimann (1951) stated that it is probably that such hydrolysis is only necessary for transport and that, for primary activity, the ester is active per se. McIlrath and Ergle (1953a) found a naturally not occurring auxin from the cotton plants which have been treated with 2,4-D, but could not prove that such substance is applied 2,4-D itself.

Watson (1948) investigated that a series of types of injury, as a result of treatment with 2,4-D, grading from that found in the first leaf to the most severe and then on to the least severe or normal is consistent for all bean planes tested, and McIlrath and Ergle (1953, and 1953a) studied similar injury effects of 2,4-D in cotton. But, they have not found in their experiments the formation of uni- or multi-foliate foliage leaves which have normal anatomical structure, the change of situation of stipules and the induction of united petiole wrapping up the shoot apex. This discrepancy may result from different agings of plants to which the chemical was applied; that is, only the cotyledons play probably different rôle from the treated bud or shoot, and consequently, induce some stages of the evolutionary sequence of shoot-formation in Leguminosae that Maekawa (1955) proposed according to 'leaf-class' concept.

4. Examination of influences of mal-formed first foliage leaf to the formation of following successive leaves. It has been demonstrated in above chapters that the cotyledons, pretreated with the vapour of methyl 2,4-

dichlorophenoxyacetate at the dormant seed-stage, influence to the morphogenesis of successively formed organs in the later development, and that methyl 2,4-chlorophenoxyacetate or the stimulus thereformed can persist for a long while in plants. The present subject is to resolve the question of where the stimuli, which induce the modification of second, third, and fourth foliage leaves, come from. There are two possibilities: this stimulus comes from the pretreated cotyledons, or from the mal-formed first foliage leaves. To make clear on this point, the following four experimental groups were set up as designed in table 5, and then the form of second, third, and fourth foliage leaves was examined during the development. The data obtained show no effect of malformed first foliage leaves to the induction of following modification in table 5.

Table 5. Leaf-formation after removal of first foliage leaves at 6-day growth period in bean seedlings with cotyledons pretreated with methyl 2,4-dichlorophenoxyacetate at dormant seed-stage.

DESIGN OF EXPERIMENT	1. Application with the chemical		treated	treated	un- treated	un- treated
	2. Removement of first foliage leaves		removed	intact	removed	intact
RESULTS	2nd foliage leaf	unifoliolate	17	15		
		difoliolate	2	2		
		trifoliolate		1	19	20
	3rd foliage leaf	trifoliolate	5	5	18	20
		tetrafoliolate	7	8	1	
		pentafoliolate	7	5		
	4th foliage leaf	trifoliolate	14	10	19	20
		tetrafoliolate	1	2		
		pentafoliolate	3	4		
		hexafoliolate	1	1		
		heptafoliolate		1		
TOTAL NUMBER OF SAMPLES			19	18	19	20

The difference between seedlings with first foliage leaves and those without such leaves, whether or not the plants were pretreated, was not significant, and contrarily, the pretreated seedlings had significant difference to untreated controls whether or not the plants maintained their first foliage leaves. It would appear from this fact that malformed first foliage leaves of treated bean were not necessary to induce the gross responses in successive trifoliolate foliage leaves, and therefore, such modification is also a result of the stimulus produced in affected cotyledons.

5. **Rôle of seed-coats in application with methyl 2,4-dichlorophenoxyacetate to intact seeds.** From above experimental results, it would seem that, when dormant seeds were exposed to the vapour of methyl 2,4-dichlorophenoxyacetate for several long hours, the chemical hardly penetrated into the inner parts of the seeds. Therefore, the chemical applied to intact seeds was expected to be absorbed in that seed-coat or cotyledons. An attempt was made to resolve the question of how much the chemical was absorbed in the seed-coat of the treated bean seed. The seeds, to which the

vapour of the chemical has enough exposed, were soaked for 0, 90 minutes, or 20 hours in running tap water respectively. When the soaking was finished, halves of these members of swelling seeds were removed their seed-coats in each experimental series, and another halves were maintained their seed-coats as they were. Thus, six experimental series were set up, and they were sown respectively in the soil of pots, and grown in greenhouse under usual condition. The mode of morphogenesis was investigated for these six series, and the data obtained here were presented in table 6.

Table 6. Soaking and removal of seed-coat pretreated with vapour of methyl 2,4-dichlorophenoxyacetate. Data are represented by the number of plants that showed each pattern.

DESIGN OF EXPERI- MENTS	Experimental series	I	II	III	IV	V	VI
	1. Duration of soaking in running tap water	0 min.	0 min.	90 min.	90 min.	20 hrs.	20 hrs.
RESULTS	2. Seed-coat of treated bean	Main-tained	Remo-ved	Main-tained	Remo-ved	Main-tained	Remo-ved
	Rate of germination (germinated/not)	19/1	10/0	20/0	9/1	20/0	10/0
	Delay in development	appeared	not	not	not	not	not
	Malformation of first foliage leaves: in both in either in neither	19	2 4 4	4 9 7	2 3 4	2 13 5	3 4 3
	Leaf-form of 2nd foliage leaf: unifoliolate difoliolate false trifoliolate normal trifoliolate	8 3 3 5	1 1 8	 1 19	1 1 2 5	 2 2 18	 10
	Leaf-form of 3rd foliage leaf: trifoliolate multifoliolate	9 10	9 1	18 2	7 2	18 2	9 1
	Leaf-form of 4th foliage leaf: trifoliolate multifoliolate	6 13	10	17 3	9	19 1	10
	Configuration of stipules and stipella to 2nd foliage leaf: associated leaves* combined leaves* simple leaf with stipule* simple leaf without stipule	4 9 5 1	10	19 1	5 3 1	18 2	9 1
	Anatomical modification in transitional region between stem and root	occurred	No	No	No	No	No
	Total number of samples in each series	19	10	20	9	20	10

* See explanation of these patterns; Furuya, 1953, Furuya and Osaki, 1955.

The data given here showed the facts that the great part of absorbed chemical is in the seed-coat. It was supposed that the diffusion of the chemical from seed-coat to cotyledon begin to take place parallel in the absorption of water in germination, and consequently, the chemical, that penetrated so, would affect to the metabolism in the germinating cotyledons.

When we had a glance at only table 6, we may expect to the seed-coat as an inducer of such formative responses as described in previous paper in lieu of cotyledons. Of course, when intact bean seeds are exposed to the chemical, seed-coats play an important rôle for the induction of the formative responses as a 'reservoir' of this stimulating substance. But, such formative responses could occur in the seedlings, when a necessary amount of the chemical applied to cotyledons, unless seed-coats were maintained. The bean seeds, which were immersed in solution of 5 to 100 p.p.m. 2,4-dichlorophenoxyacetic acid for a short time, could also represent same responses as described in the first paper of this series though the seedling did not maintain their seed-coat (Furuya; 4th paper of this series, in press). It was concluded from these facts that the organ, which is requisite to occur such formative effects in the seedling, is not the seed-coat but the cotyledons.

Some dicots show noticeable depression in germination of seeds after treatment with methyl 2,4-dichlorophenoxyacetate (Mullison and Hummer, 1949), but, on the other hand, the bean used in previous and present work did not show no influence of treatment on the rate of germination. The results of chapters 2 and 5 suggest that such different rates of germination may be explained by whether or not the chemical could penetrate into the tissue of embryo through the seed-coat.

Summary

A number of experiments were made to find the answer to the question of whether the vapour of methyl 2,4-dichlorophenoxyacetate applied to dormant bean seeds may stimulate directly each organ for the duration of treatment, and, further, of whether the induced formative effects in the bean seedling as presented in the first paper of this series (Furuya and Osaki, 1955) will result from that only the cotyledon is affected and then works as the source of secondary stimulus to the seedling.

1. Any influence was not observed in the growth and behaviour of decotylated embryos, which were excised from bean seeds treated with the vapour of methyl 2,4-dichlorophenoxyacetate for five weeks, throughout the seedlings in sterile culture *in vitro*.

2. Decotylated embryos, that were excised aseptically from untreated seeds and exposed to the vapour of the chemical for several long hours, showed a wide variety of patterns of formative effect in the course of *in vitro* development, but represented no response as similar to those induced in the seedlings with cotyledon treated with the chemical at the stage of dormant seed. In this case, the degree of severity in formative effects was proportional to the duration of exposure.

3. The correlation between the occurrence of formative effects to seedling and the duration of maintenance of cotyledons was studied in pretreated plants. Such formative responses have required the different durations of maintenance of cotyledons on seedling respectively.

4. Malformed first foliage leaves, produced as a result of application with the chemical for seed-stage, have not played any rôle to induce uni- or multifoliolate foliage leaves in the successive foliage leaves.

5. When the dormant bean seeds were exposed to the vapour of the chemical, the great part of the chemical was absorbed in the seed-coat, and no or few chemical penetrated into the inner part of seed. Thus, the seed-coat was regarded as a reservoir of the chemical in this treatment, but did not play any essential rôle, such as cotyledons, for the induction of formative effects.

It is concluded from above results that in the growing points of seedling the formative effects, following treatment to intact bean seeds, could be induced only by the stimulus through the treated cotyledons, but not induced directly by the chemical itself to each meristematic cells, or tissue, at the time of treatment.

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Effects of the Vapour of Methyl 2,4-dichlorophenoxyacetate on Growth and Differentiation in *Phaseolus vulgaris* L.

III. The Distribution of Thiamine in the Seedlings

By

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Introduction

It was previously described by Furuya and Osaki (1955) that the seedlings derived from the seeds having been treated with the vapour of methyl 2,4-dichlorophenoxyacetate show various morphological abnormalities through the course of their development. Though mechanism of the action of this chemical is still unknown, there have been obtained some proofs for the phenomenon that the stimulus of this chemical is given to the cotyledon directly, and then secondarily transferred to the remaining parts of the embryo during the germination (Furuya, 1956).

Thiamine is acquainted as a main component of co-carboxylase. Supplied to the plants exogenously, this was reported having auxin-like activities in some cases (Leopold and Guernsey, 1954), and having anti-auxin-like in the other (Kandler, 1953). Recently such conclusion that the naturally occurring auxin, indole-3-acetic acid, plays an important rôle in diverse steps of differentiation of the plants becomes more and more affirmative. But that the substances besides the indole-3-acetic acid, e.g. other auxins, vitamins, nucleic acid constituents, take parts in, is also demonstrated, and it seems to be rather appropriate to consider that the balances of and the interactions between these substances are most essential factors in the analysis of the phenomena of differentiation and development of the plants.

Using the macrochemical method together with the histochemical one, we have been examining the changes of distribution of thiamine in the seeds and the seedlings of bean plant, and the results were published elsewhere (Chikubu et al. 1955, 1956). According to these, thiamine is concentrated in the cotyledon of dormant dry seeds, and as the germination proceeds, it is translocated from the cotyledon to the remaining parts of the seedlings. In this study, our special attentions are paid on, whether the pretreatment with the vapour of methyl 2,4-dichlorophenoxyacetate has any influence upon the rate of thiamine translocation, and upon the patterns of distribution of thiamine in the various parts of the germinating bean plants. And also, the comparison is made on the thiamine contents in the seedlings grown under the light condition and in the dark.

Materials and Methods

Phaseolus vulgaris L., clone Master Piece, was exclusively used. After sterilization with 0.15% solution of Uspulun for 20 minutes and soaking in running tap water for 20 minutes, the seeds were placed on the moist sand, and allowed to germinate at 25°C. in the dark. The method of treatment with the vapour of methyl 2,4-dichlorophenoxyacetate to dormant dry seeds accords with that described previously (Furuya and Osaki, 1955). Effects of the vapour were, in this case, somewhat weakened as a result of rinsing out a trace of sterilizer with running tap water (cf. Furuya, 1956). When grown under the light, the pots in which the plants were germinating were set in the green house under the daily sunlight.

The investigation was carried out over a period of three years extending from August 1953 to July 1955. In order to detect the distribution of thiamine, both the macrochemical and the histochemical methods were employed.

Macrochemical — To analyse a pattern of distribution of thiamine, the plant body is separated into 5 parts, i.e. cotyledon, first foliage leaf with shoot apex, hypocotyl, transitional region, and root, as shown in Fig. 1. The

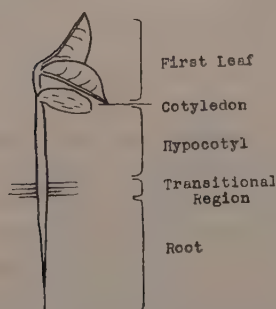


Fig. 1. Each part of seedling of *Phaseolus vulgaris*.

transitional region is a region in which a recombination of vascular system between stem and root occurs, and should be discriminated from other parts. But in the case of macrochemical analysis, the transitional region and the root are examined together for the technical convenience. The fresh weight of each parts of the seedlings at different growth periods is listed in Tables 1 and 2.

Thiochrome procedure depends upon the oxidation of thiamine to thiochrome, which fluoresces in ultraviolet light. The standard method as described in "Method of vitamin assay" of the Association of Vitamin Chemists, Inc., was im-

Table 1. Changes of fresh weight during germination in the dark.
mg./individual part. t: treated. c: untreated control.

Parts	Application with the chemical to seeds	Growth period : days				
		0	2	4	7	14
Cotyledon	t	390	650	650	500	380
	c		640	650	395	293
First foliage leaf	t	1.10	3.80	10.1	85.0	115
	c		6.80	31.1	72.2	—
Hypocotyl	t	3.42	19.2	100	1415	2980
	c		40.0	781	1946	—
Transitional region and root	t	0.67	12.9	126	330	833
	c		33.3	215	335	—

proved by Fujiwara (1949), in order to purify a sample solution more easily, using an ion exchange resin, Parmitit. The principles of procedure are as follows.

Table 2. Changes of fresh weight during germination in the light.
mg./individual part. t: treated. c: untreated control.

Parts	Application with the chemical to seeds	Growth period: days			
		0	3	7	14
Cotyledon	t	39.0	725	750	275
	c		750	365	300
First foliage leaf	t	1.10	5.63	36.0	1008
	c		14.0	825	1158
Hypocotyl	t	3.42	22.7	130	910
	c		160	1040	600
Transitional region and root	t	0.67	10.0	235	463
	c		104	555	504

Accurately pipetted amount of suitable prepared sample, buffered at pH 4.5, is heated for 30 minutes in a boiling water bath with occasional shaking. After cool, for estimation of total thiamine, added a freshly prepared Taka-diastase solution to sample, and incubated at 38°C overnight. Sample solution, then, centrifuged, and passed through a column of Parmitit at a speed of 1 drop per 3 seconds. Turning out of thiamine from a column of Parmitit is achieved by passing of just boiling 25% KCl solution through a column. To convert thiamine to thiochrome, a fresh mixture of 3:1, 1% $\text{K}_3\text{Fe}(\text{CN})_6$ aqueous solution and 25% NaOH, is added to sample solution. Thiochrome produced is extracted with iso-butyl alcohol, and its concentration is determined by its strength of fluorescence under the ultra violet light comparing with that of control.

Histochemical — Method to determine the localization of thiamine microscopically was formerly used by Somers et al. (1945 and Simpson (1951) mainly working with cereals. As to the reliability of this method, Araki (1950) found good reasons to believe that the induced fluorescence is that of thiochrome. But in the case of plant cells, some doubts remain, since the existence of various kinds of fluorescence, which disturbs the detection of thiochrome, was reported. An example of such substances is that of cellulose, a main component of the cell membrane characteristic to the plant cells, because after the suitable degradation this produces a fluorescence having a color resemble to that of thiochrome. To remove such disturbing fluorescent substances, the tissue slices were immersed before the oxidation in organic solvents, which do not resolve thiamine, such as buthanol, ethanol, benzol, xylol, ether, acetone, chloroform, etc. According to our experience, chloroform has given best result.

Our schedule used is that of Chikubu et al. (1953). Fresh tissue slices or those fixed by absolute alcohol or benzol were covered with fresh mixture

of equal parts of 30% NaOH and 4% $K_3Fe(CN)_6$ aqueous solution for a few minutes. Then, slices were washed with distilled water two or three times quickly, and after excess water was removed, slices were mounted in glycerin, which does not contain any disturbing fluorescent substance. Ultraviolet light produced by Mazda mercury lamp was transmitted through a filter (equals to Wratten No. 18A), and projected on the treated slices obliquely. Then, the induced fluorescence was again transmitted through filters (equal to Wratten No. 8 and No. 39) set in the tube of microscope, and get to the surface of photographic film.

Results

Macrochemical — In Figs. 2 to 5, the results of macrochemical determination of thiamine contents under dark condition are summarized respectively. The difference between the changes of thiamine contents in the cotyledon of the treated plants and these of the controls is not so conspicuous (Fig. 2). In the control plants of 2-day-old, the total content of each

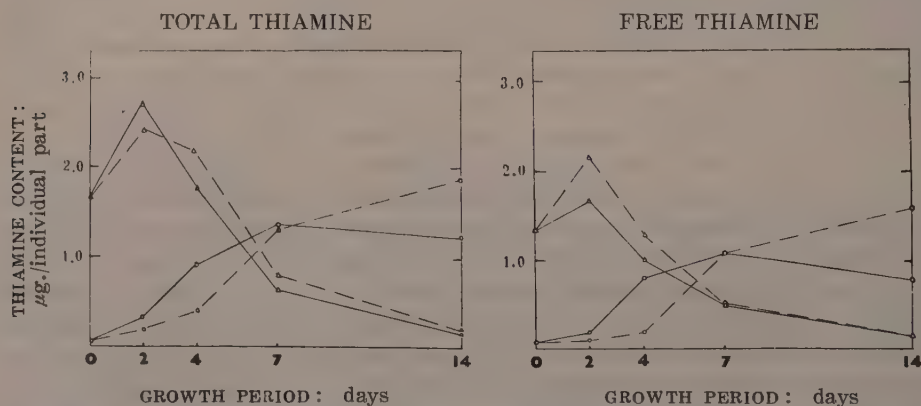


Fig. 2. Thiamine content in cotyledon (Δ) and decotylated embryo (○) grown in dark condition. — — —, treated; — — —, control.

cotyledon increases from 1.69 μ g of the beginning up to 2.72 μ g, and also in the case of treated plants, the same increase can be presumed. As to the decotylated embryo, the rate of increase of thiamine content is somewhat slower than that of the control, but at the growth period of 7-days the levels of the contents of both are almost the same, and thereafter, the curve of the treated is still upward, while that of the control is downward. In the case of free thiamine, the similar behavior of thiamine contents is observed.

In the first foliage leaf and hypocotyl, there is a common tendency as to both free thiamine and total, that the rate of increase of thiamine in the treated plants has a delay of 1.5-2.0 days from that of the control ones (Figs. 3 and 4). On the other hand, the reverse tendency can be seen in the case of transitional region between stem and root (Fig. 5). Within a growth period of 4-days both curves of the treated and the control plants have

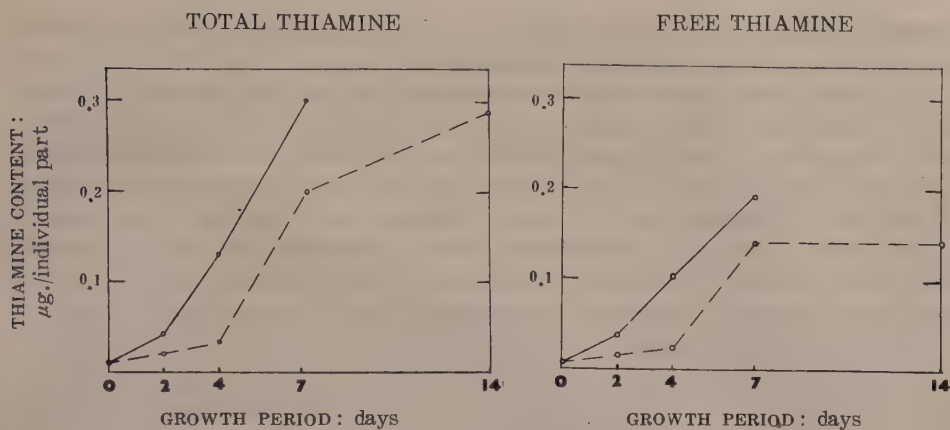


Fig. 3. Thiamine content in first foliage leaves. Dark condition.
— — — treated; — control.

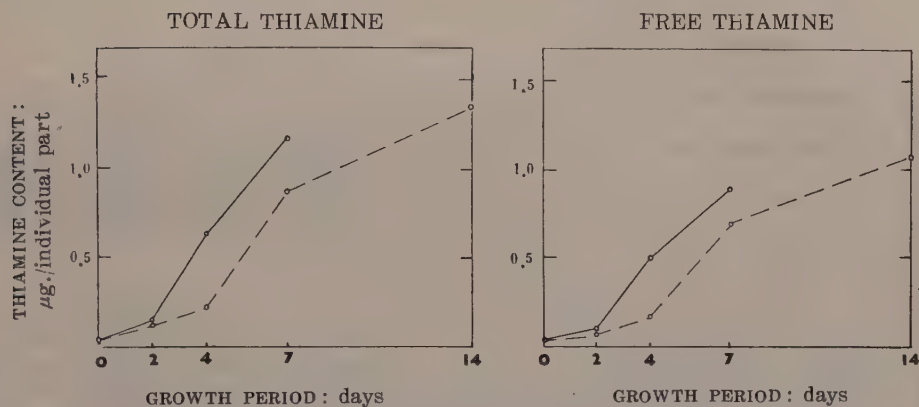


Fig. 4. Thiamine content in hypocotyl. Dark condition.
— — — treated; — control.

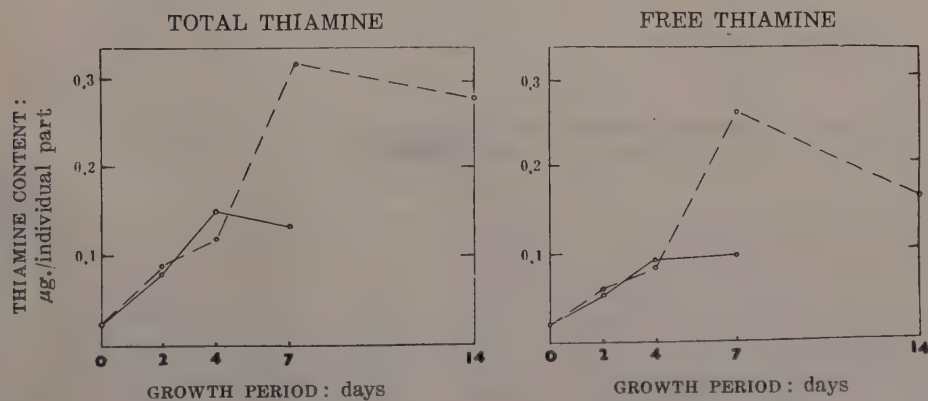


Fig. 5. Thiamine content in transitional region. Dark condition.
— — — treated; — control.

same trend, but after this time, the rate of the increase in the free thiamine and the total increases those of the controls.

The data were recalculated on the basis of $\mu\text{g.}$ per 100 g. dry tissue, and arranged in Table 3. The behaviors of thiamine in the cotyledon are quite the same in the treated plants and the control. In the first foliage leaf, the treated plant of 2-day-old has less thiamine, but in the hypocotyl, the content of thiamine in the treated plants of 4-day-old is almost 3 times of the control, and in the transitional region, the treated plants always maintain higher level of thiamine content than the control. The transitional region and root are separately tested in the treated plants (Table 4).

Table 3. Changes of thiamine concentration (mg. per 100 g dry tissue) in each fraction of the seedlings grown in the dark. t: treated. c: untreated control.

	Parts	Application with the chemical to seeds	Growth period: days				
			0	2	4	7	14
Total thiamine	Cotyledon	t		0.74	0.59		0.18
		c	0.48	0.93	0.66	0.35	0.25
	Decotylated embryo	t		3.14	2.51	1.19	0.85
		c	1.59	3.72	1.26	0.79	0.60
	First foliage leaf	t		1.49	2.13	1.73	1.74
		c	1.04	2.23	2.18	1.51	—
	Hypocotyl	t		3.54	3.72	1.31	0.97
		c	1.24	3.05	1.28	1.44	—
	Transitional region and root	t		2.38	1.45	1.44	—
		c	4.34	1.40	0.71	0.61	—
Free thiamine	Cotyledon	t		0.63	0.40	0.27	0.18
		c	0.40	0.61	0.39	0.31	0.20
	Decotylated embryo	t		1.78	1.50	0.94	0.76
		c	1.48	1.53	1.06	0.60	0.36
	First foliage leaf	t		1.25	1.50	1.26	1.02
		c	0.74	1.50	1.56	0.92	—
	Hypocotyl	t		2.14	3.01	1.00	0.75
		c	1.13	1.77	0.94	1.11	—
	Transitional region and root	t		1.72	1.10	1.31	—
		c	3.29	0.96	0.44	0.39	—

Table 4. Changes of thiamine concentration (mg. per 100 g. dry tissue) in transitional region and root of treated plants.

	Parts	Growth period: days				
		0	2	4	7	14
Total	Transitional region	2.58	2.12	1.30	1.98	2.12
	Root	5.36	3.75	2.08	0.91	0.57
Free	Transitional region	3.11	1.48	1.02	1.97	1.06
	Root	4.20	2.89	1.42	0.77	0.34

As the seedlings develop, the total thiamine content of the whole plants grown under the dark is decreased, while that grown under the light increases evidently (Fig. 8). But in the case of free thiamine we cannot observe such a difference as seen in the case of total thiamine. Both fate of free thiamine in the plants grown in the dark and under the light have similar tendency. As mentioned in the chapter of consideration, the difference between the content of free and total thiamine is thought as an ester form. Then, such changes of amount of total thiamine come from those of its ester form. Especially, remarkable modification is observed in the first foliage leaf. Comparing Fig. 3 with Fig. 6, the total thiamine content in the leaf of control plant grown in the light attained to $2.11 \mu\text{g.}$ per each organ, but in the dark only to $0.13 \mu\text{g.}$, probably due to its etiolation in which the growth and differentiation scarcely occurred. The rate of increase of total thiamine content in the first foliage leaf of treated plant grown in the

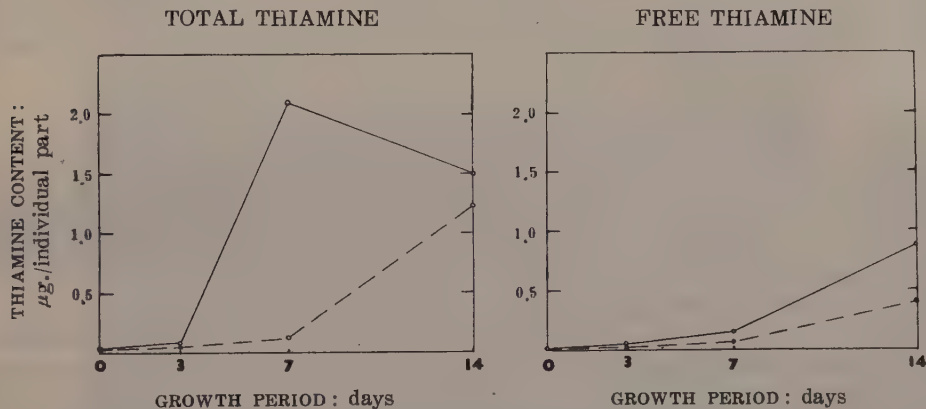


Fig. 6. Thiamine content in first foliage leaves. Light condition.
— — — treated; — control.

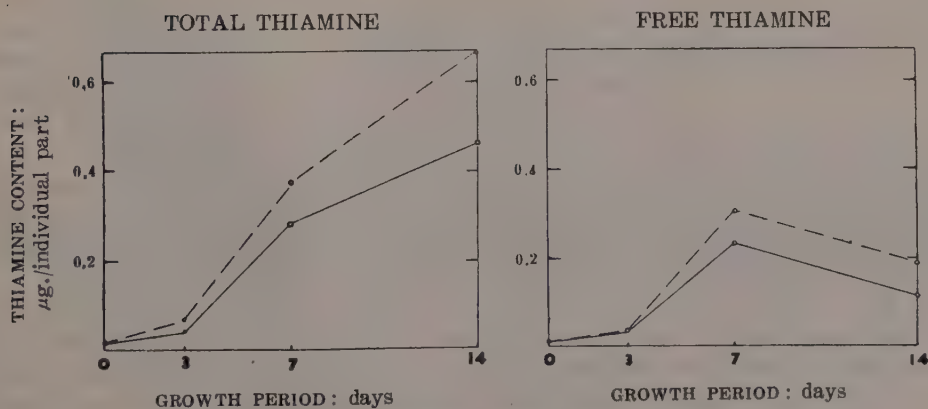


Fig. 7. Thiamine content in transitional region. Light condition.
— — — treated; — control.

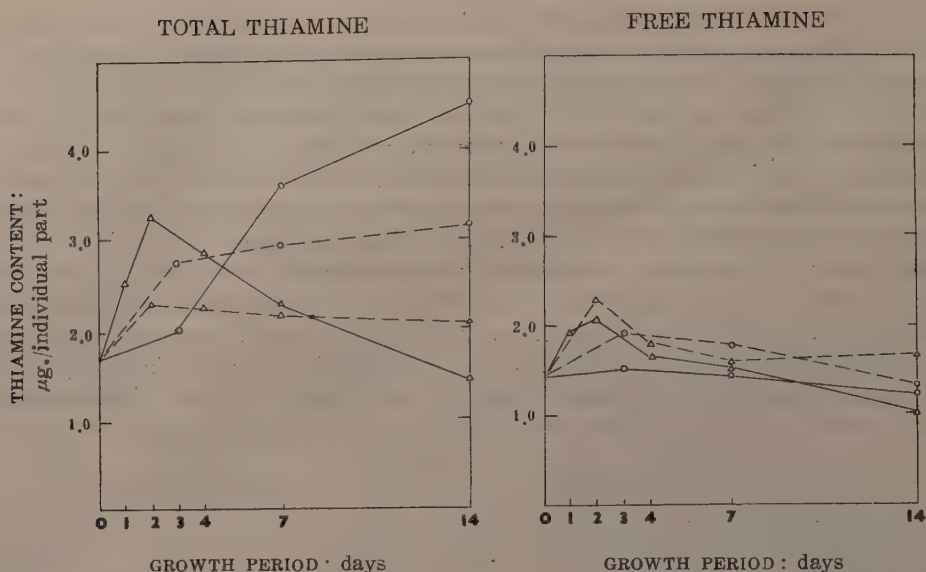
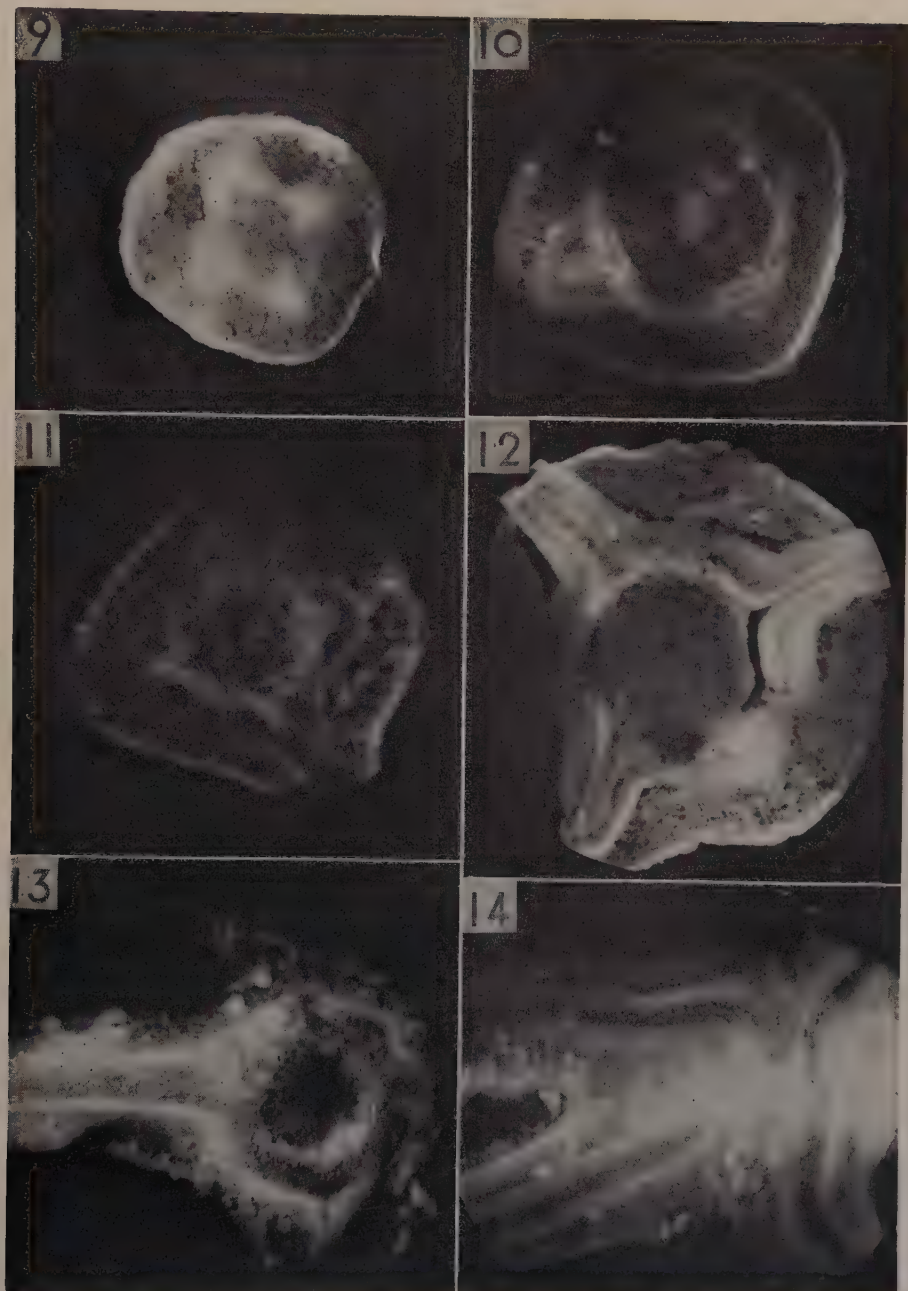


Fig. 8. Thiamine content in whole plant. (o) Light condition. (Δ) Dark condition. — — — treated; — — — control.

light is exceedingly slower than the control in the light, and at a growth period of 7-days, the level is maintained at $0.13 \mu\text{g.}$, but increases to $1.18 \mu\text{g.}$ at a growth period of 14-days. As regard to the free thiamine, the rate of increase is slower than the control, but comparing with that in the dark it is very high. In the transitional region between stem and root, the content in the dark seems to decrease (Fig. 5), but in the light it still continues increasing (Fig. 7).

Histochemical — — In the transitional region between root and stem of the seedling pretreated with the vapour of methyl 2,4-dichlorophenoxyacetate at dormant seed-stage, a conspicuous shoulder-like organ is formed. This shoulder is initiated by the proliferation of the cells existing in the procambium in the embryo, and the thiochrome fluorescence is mainly localized in these actively dividing cells. As the plant grows, the tissues also differentiate, and it becomes clear that this shoulder is anatomically the same with the normal adventitious root. And in this region, the thiochrome fluorescence is concentrated to the meristematic tissue of the tip of shoulder, the cambium, and the phloem tissue (Figs. 9-14). In xylem tissue, thiochrome fluorescence is not observed, and in stead white or brownish white disturbing fluorescence can often be seen.

It is known that the differentiation in the mesophyllous tissue in the first foliage leaf does not occur as a result of vapour treatment. Since the removal of the disturbing fluorescence from the leaf is still insufficient, we cannot get a good photograph. In the treated plants and the control plants of 3-4 days old, there is no visible difference as to the localization of thiochrome fluorescence in the first foliage leaf, and thiochrome fluorescence



Figs. 9-14. Cross sections of transitional region in treated plants of various ages.—Fig. 9. 2-day-old plant.—Fig. 10. 3-day-old plant.—Figs. 11 and 12. 6-day-old plant.—Figs. 13 and 14. 14-day-old plant. Showing the development of the fin-like organ and related distribution of thiochrome fluorescence.

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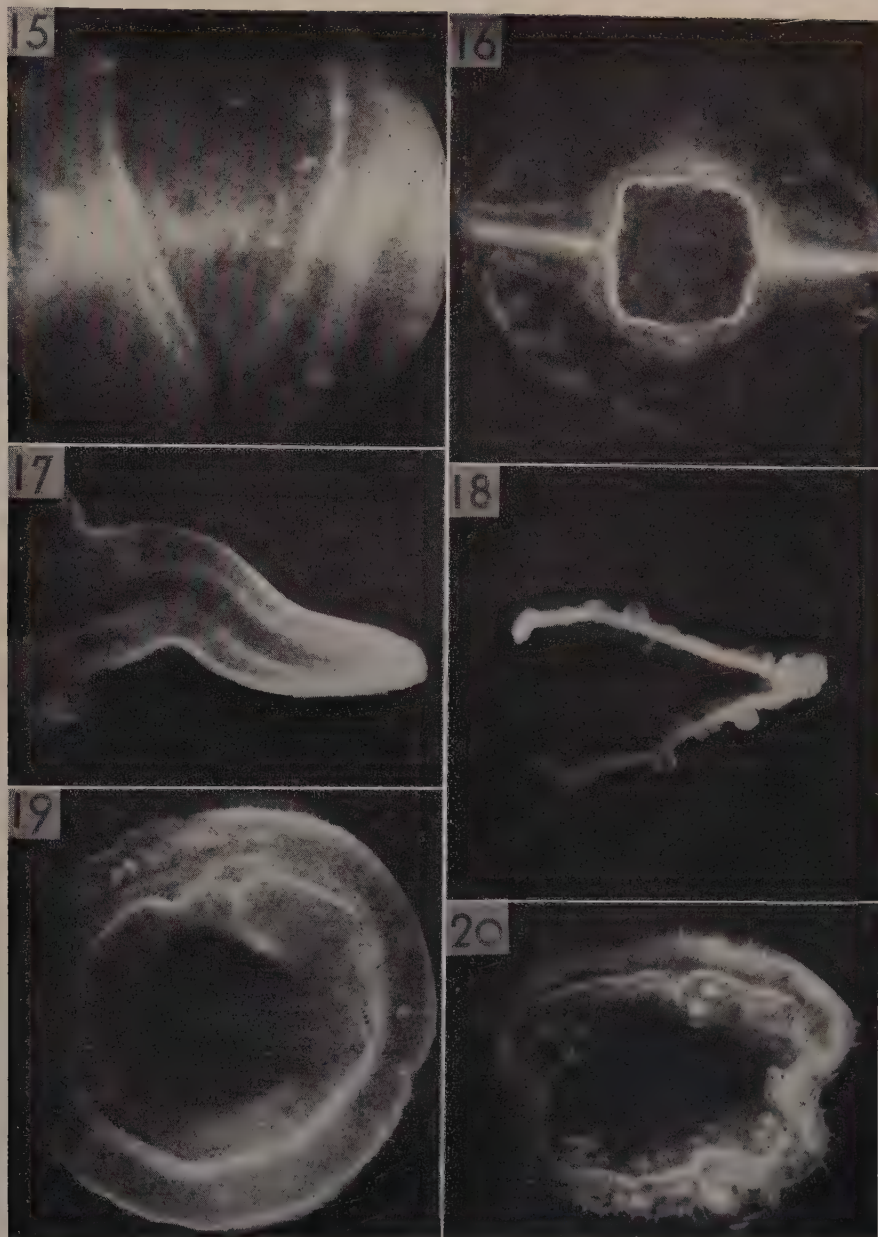


Fig. 15. Longitudinal section of transitional region of treated plant 7-day-old. — Fig. 16. Cross section of transitional region of control plant 7-day-old. Compare with Fig. 12. — Fig. 17. Cross section of the shoulder. Treated plant 14-day-old. — Fig. 18. Cross section of first foliage leaf of treated plant 6-day-old. — Fig. 19. Cross section of lower part of hypocotyl. Treated plant of 14-day-old. — Fig. 20. Cross section of epicotyl of treated plant 14-day-old.

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is always distributed uniformly in the mesophyllous tissue. But, thereafter, the first foliage leaf of the treated plant begins to take a characteristic form, and a part of mesophyllous tissue has a strong fluorescence, and the other part loses this, remaining a strong red-brownish disturbing fluorescence (Fig. 18). Whether this differentiating localization of thiochrome fluorescence correlates with the anatomical modifications of first foliage leaf, cannot be verified. The same phenomenon, the partial disappearance of the thiochrome fluorescence, is also observed in the old first foliage leaf of the control plant.

In other parts of the treated plant, visible anatomical abnormalities cannot be noticed within a growth period of 14-days. And the distribution of the thiochrome fluorescence in the seedlings is restricted to such tissues as meristematic tissues, cambium, phloem both in the control and the treated plants (cf. Chikubu et al. 1955, 1956).

Considerations

It can not be expected that there is any direct interaction between the action of methyl 2,4-dichlorophenoxyacetate and the content of thiamine, but under the following presumption, we conducted our experiments, namely, if the chemical influences upon the cotyledon, some modifying effects may occur in the course of translocation of various substances from the cotyledon to the embryo. Because, it is generally accepted that, during germination, the cotyledon of bean plant is an important reserve organ, and almost all of the materials needed for the primary growth of the seedlings is supplied from the cotyledon.

We cannot point out the remarkable difference on the fate of thiamine in the cotyledon between the treated plant and the control. According to Furuya (1956), however, the movement of the affected principle from the cotyledon is achieved within first 24 hours after starting germination, then we can imagine it probable that there exists a delay of the rate of increase of thiamine in the cotyledon of the treated plants during the first 24 hours. Accordingly, an analysis in this duration should be tried in more details.

The phenomenon that the content of both total thiamine and free increase in the cotyledon after germination under dark condition is very interesting, because the cotyledon is commonly thought to be an organ functioning decompositively during the course of germination. Working with mung bean, Simpson et al. (1953) concluded that, the plants growing in the dark utilize thiamine stored without its synthesis and under the light with its synthesis. And also, Burkholder et al. (1945) pointed out the constancy of the total thiamine level in the leguminous seedlings grown in the dark. But our results indicate a possibility that thiamine may be synthesized in the cotyledon in the dark, and moreover, that the other active substances also may be synthesized, e.g. riboflavin (Simpson et al. 1953). A doubt remains, however, whether thiamine is actually synthesized, or thiamine exists in an undetectable form for our present method of analysis at the dormant seed stage.

As regard to the increase of thiamine content in the hypocotyl, on $\mu\text{g.}$ per individual basis, we can detect a delay of about 2 days in the treated plants, and an advance on the dry weight basis. This fact is explained as the growth of hypocotyl of the treated plant is inhibited, but we cannot observe any visible morphological abnormalities, though such modification of thiamine content occurs.

At a growth period of 7-days, the rate of increase of thiamine content in the transitional region of the treated plants exceeds suddenly that of the control. On the dry weight basis, the treated plant always exceeds the control. We have no datum in the case of bean plant, but it is reported that, for the growth of root of pea plant, thiamine is a necessary substance, especially for the maintenance of the meristematic activity in the root tissue (Adicott 1938). The shoulder, produced as a result of the treatment, is thought to be synonymous with the vertically continuous production of the lateral roots. Then, the increased amount of thiamine may be needed to keep the activity of the increased mass of the meristematic tissue. There remains a question, however, why the shoulder, a clump of the lateral roots, does not make further growth, in spite of having sufficient amount of thiamine. Perhaps, we consider, some substances other than thiamine concern in this step, and are too large or too small in quantity to advance the further growth of the lateral roots.

Thiamine concentration in the first foliage leaf increases even in the dark, but its amount jumps up when the light is supplied to the plant. In the cotyledon, hypocotyl and transitional region, there appeared a little difference in the thiamine content between the plant grown under the light and in the dark, but the difference is much remarkable in the case of first foliage leaf, since the content of the control plant aging 7 days under the light is over an amount of 7 times of that in the dark. In general, the difference of quantity between the free and the total thiamine is accepted as a quantity of ester (bound) thiamine. According to this, the content of ester thiamine is extraordinarily high in the first foliage leaf of 7-day-old plants supplied with light. When 7 days passed since germination began, the first foliage leaf just matures, and this just matured leaf under the light condition is known to be rich in various functions such as photoperiodic response, etc. Then, it is reasonable to consider that thiamine, especially its ester type is most vigorously produced in the leaf just expanded under the light. Bonner (1942) also pointed out that thiamine is synthesized in the leaf of tomato plant under the light. As to the rate of production of thiamine in the leaf, we can recognize a considerable difference between the treated and the control plants. When the treated plant is grown under the light, morphological abnormalities of the leaf are more marked. And the total amount of thiamine in the leaf of the treated plants aging 14 days, is only about 50% of the control, while the leaf is fully expanded at this time. The loss of differentiation in the mesophyllous tissue of the treated plants may cause the lowering of function to synthesize thiamine.

Thus, it would be possible to say on the rôle of thiamine during the

germination of bean plant. As far as we have observed, thiamine has not direct relation with the phenomena of the differentiation, and is supplied to the tissue containing actively dividing cells from the other parts of the plant in order to maintain the youthfulness of such cells. Whether the treatment has any influence on the rate of the fate of thiamine from the cotyledon, cannot be determined yet, because the effect is somewhat weakened for the reason that the seeds are rinsed before sowing, but it may be said that the pattern of distribution of thiamine is considerably influenced.

Summary

1) The distribution of thiamine in the seedlings of *Phaseolus vulgaris* L., "Master Piece", developed under both light condition and dark after treatment with the vapour of methyl 2, 4-dichlorophenoxyacetate at seed stage is examined both macrochemically and histochemically.

2) From the results of our observation, we cannot yet conclude that the treatment causes the marked influence upon the thiamine translation from the cotyledon to the remaining parts of the embryo. But as to the rate of the distribution of thiamine to each part of the plant, i.e. the first foliage leaf, the hypocotyl, and the transitional region between stem and root, the treatment has some influences.

3) Within 2 days after germination, the thiamine content in the cotyledon rises not only under the light but in the dark.

4) Thiamine is concentrated mainly in the tissues containing actively dividing cells. Direct relationships of thiamine to the formative effects cannot be concluded.

5) When the light is supplied to the plants, thiamine is most vigorously synthesized in just expanded leaves.

We wish to thank Prof. Fumio Maekawa for his helpful comments during this investigation, and also to express our deep appreciation to the laboratory staff of the Food Research Institute for their generosity of giving facilities to use their equipments.

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